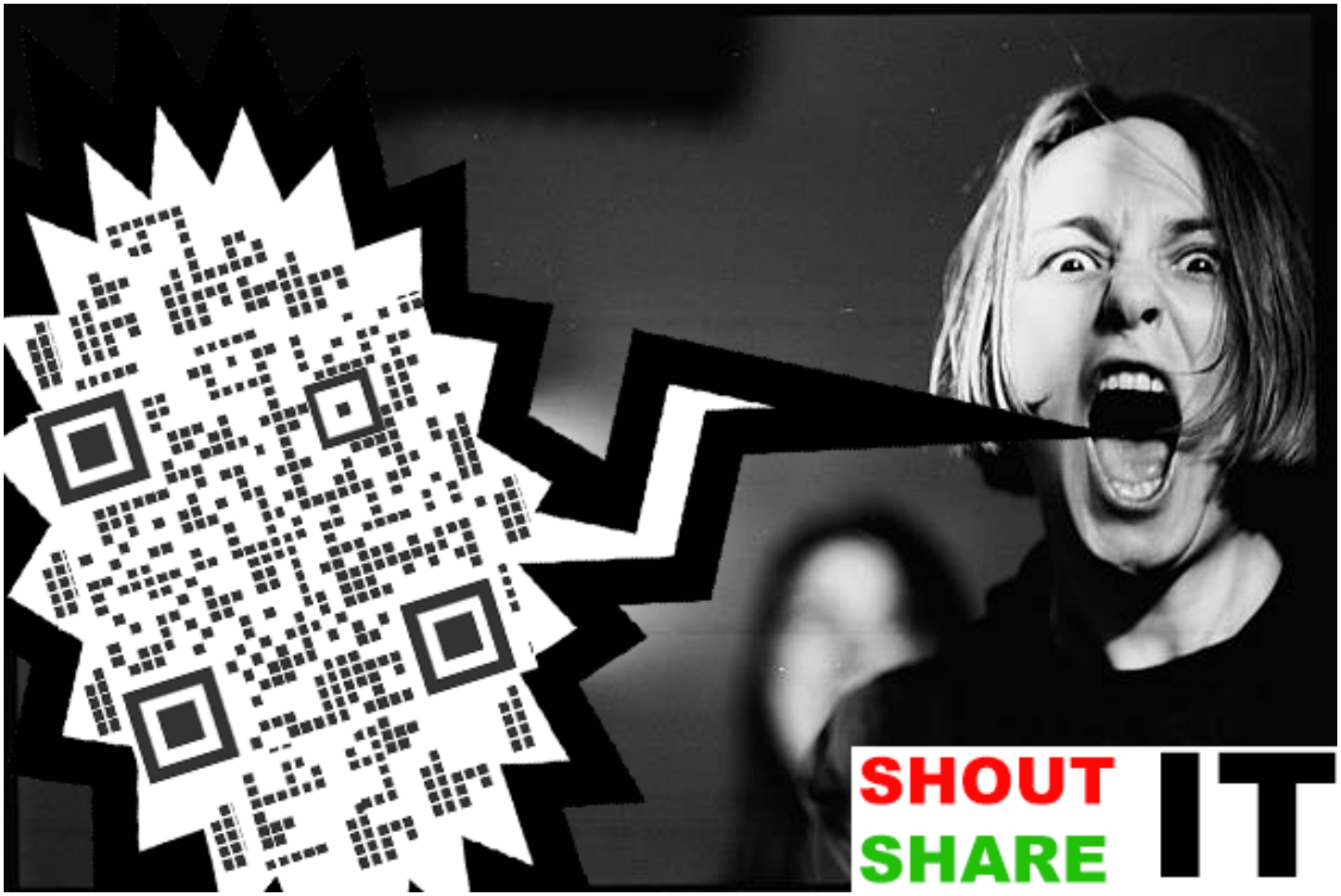
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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

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BIOLOGICAL BULLETIN.

MATURATION OF THE EGGS OF DIEMYCTILUS TOROSUS.

DR. HECTOR LEBRUN, Brussels, Belgium.

During my stay in California, where the Belgian government sent me to verify the results of Dr. Eisen's investigations on the testicles of the Batracoceps, and to study the eggs of these and other Batrachians, the occasion also offered itself to study the maturation process in *Diemyctilus torosus*.

I knew, by the work of Professor Ritter, of the University of California, that the copulation of this species takes place during the months of March and April, and therefore immediately devoted my investigations to this subject. My material came from Tocoloma, Marin County, California, where Professor Van der Naillen called my attention to a large quantity of specimens. In my previous publications, I have shown it to be absolutely necessary to have the largest possible number of specimens on hand to have good material in the different stages of the maturation of the eggs, because these phenomena last only a few days, and during this period, it is necessary to kill a large quantity of females. All of these conditions are perfectly realized in Tocoloma.

By the kindness of the San Francisco Microscopical Society, which generoûsly opened to me its rooms and instruments, I began to study the material collected, and demonstrated my preparations at a meeting of the Society on the 15th of May.

As the present note is preliminary and a summary of the researches made in California, my investigations will be continued in Europe and published in the review (La Cellule).

The ovary of *Diemyctilus* contains from five to six species of eggs in different stages of development. I will not attempt to detail the history of these eggs from ovogony to the maturation stage, but will only state that, as a result of my investigations, I have found absolute confirmation of my researches on European urodels and on Siredon.

I will here limit my references to the process of the maturation of the egg.

When the egg is ripe, the nucleus is situated at the animal pole, voluminous, and contains four to five hundred chromatic nucleoles, in various stages of dissolution, and resolving into granula. The first appearance of maturation is the vacuolization of the karyoplasma and the dissolution of all the granular chromatin, except a little quantity of nucleoles. These have in this stage a propensity to fuse together. Shortly, the membrane of the nucleus disappears, and during this stage, the chromatic element is represented in the egg by spherical nucleoles and by masses made by fusion of other nucleoles.

All these phenomena are finished in the ovaries. When the egg is found in the peritoneum the spindle of the first polar body is finished and the chromosomes fixed on the spindle. These are formed in a small region of karyoplasma which is not invaded by the cytoplasmic inclusions after the disappearance of the nuclear membrane. There are no centrosomes, but beautiful asters. The filaments of these orders are crossed on the equatorial plane of the figure as is known, by *Batrachoceps* (Eisen), by pollen-mutter cells (Osterhout and his pupils) and tritons (Lebrun). The first figure and the expelling of the first polar body is finished in the superior part of the oviduct. The second figure runs out during the egg's passage through the oviduct to the cloaca. The chromosomes are twelve as in the other urodels. They are quadripartite as in the first polar figure and have the form of a cross, resembling those found in European tritons and called "oiselets." I will not, by this, say that I give to these forms the same signification as do Von Rath, Haecker, and others. On the contrary, I conclude that in the two figures the chromosomes divide longitudinally in the equatorial plane.

San Francisco, Cal., May 15, 1901.

NEW SPECIES OF CERATOPOGON.[1]

WILLIAM HENRY LONG, JR.

The family Chironomidæ has been much neglected by dipterologists, notwithstanding the number and diversity of its species. Even the larval forms which are especially interesting have not, as yet, received as much study as they deserve. The writer's attention was first directed to this family by finding the larvæ of a species of *Ceratopogon* in large numbers in the vicinity of Austin, Texas, during the winter months. Certain peculiarities of its development induced him to extend his study to other species of *Ceratopogon* in the same vicinity. This study brought to light several interesting facts, some of which may prove of value in separating the species of this large and complex genus. Two of the species included in this article seem to be myrmecophilous, the first, it seems, to be recorded from America.

CERATOPOGON BRUMALIS, sp. nov.

♂, length 3 mm., wing 2 x .5 mm.; ♀, length 2.5 mm., wing 2 x .75 mm. Two subcostal cells; third or cubital vein terminating distinctly before the middle of the wing with a white spot at apex of costal vein; wings and entire insect densely pilose; halteres white; metatarsi equaling or slightly shorter than the following joint: tibia of female with black lanceolate scales; last four joints of the male's antennæ longer than the basal ten joints; eleventh about 1.5 times as long as any succeeding joint. Antennæ not longer than mesonotum.

♀, head piceous; pile on tips of antennæ and palpi whitish; joints 2–9 of antennæ ovate subequal, joint 1 much larger and more globose; joints 10 to 14 inclusive ovate-oblong; apical joint prolonged into a blunt point. Each of the joints 2–9 inclusive has two large sense organs, which are curved, transparent and on upper surface of joint about 90° apart; other and much smaller sense organs occur near the apex, three or more to each joint; while joints 13 and 14 are fairly covered with minute organs of similar nature. Each joint, except the first, is pubescent and bears a circlet of bristles. Mesonotum dark brown, pruinose, pile brassy; sides of prothorax with a bunch of yellow bristles; pleuræ naked; mesopleuræ white; abdomen black, shining, pile light yellow, reddish in certain lights; venter brown; external genitalia subglobose,

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 17.

yellowish white ; legs yellowish brown, strongly infuscated ; knees yellow ; tibiæ spurred, outer edge with a row of black lanceolate scales ; bases of tarsi light yellow ; first two joints subequal, the others shorter ; each joint with a graduated row of short bristles on the plantar surface ; empodium strap-shaped, bristly-fringed, nearly as long as claws, claws subequal. Neuration of wings [1] as follows : Costal vein strong, terminating distinctly before the middle of the wing, pilose except for a small naked spot near the base ; third or cubital vein strong, terminating at apex of costal vein, with

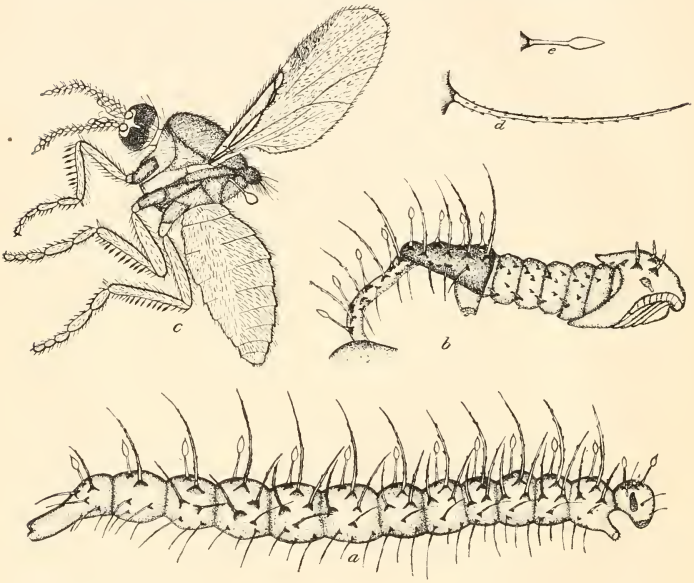


FIG. 1. *Ceratopogon brumalis*. *a*, larva ; *b*, pupa ; *c*, female imago ; *d*, lateral barbellate bristle ; *e*, dorsal ovate-lanceolate bristle.

a strong auxiliary vein extending from upper end of recurrent vein to juncture of costal and cubital veins ; nearly midway between the upper end of recurrent vein and apex of cubital, the latter vein curves forward abruptly till it touches the auxiliary vein, making two loop-like subcostal cells ; the second of which is much the larger ; subcostal vein a mere point of juncture ; recurrent vein strong, making a very obtuse angle with base of median vein, latter strong for the first third of its length, then distinct, but delicate to to margin of wing. Discal vein obsolescent before its juncture with the

[1] Nomenclature of neuration of wings after J. Winnertz.

median vein so that no prefurca is visible ; axillary vein distinct from its base to apex terminating in the posterior margin of the wing ; posterior vein short but distinct ; anal veins more or less indistinct, especially near base ; Anterior costal cell long and narrow with apex acute ; subcostal cells loop-like, first very small, often wanting, represented by the line of fusion of the auxiliary and cubital veins ; anterior cubital cell narrow, but enlarged at apex ; second cubital cell with two obsolescent veins which join near base of cell, making a V shaped figure ; between the upper anterior one of these veins and the margin of the wing, the cubital cell is densely pilose, except the small white spot at the juncture of the costal and cubital veins produced

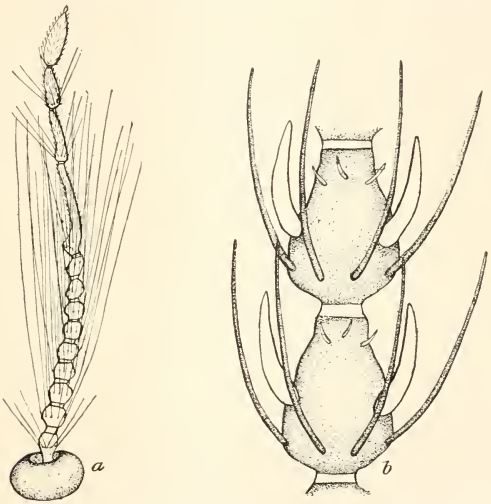


FIG. 2. *Ceratopogon brumalis*. *a*, male antenna ; *b*, two joints of female antenna showing sense organs.

by the absence of pile and by the hairs on the contiguous veins being whitish at that point. Two discal cells, subequal with an obsolescent vein in the posterior part of the second parallel with the auxiliary vein.

Coloration of male nearly the same as that of the female : differs from female as follows : Pile on entire insect longer, denser and of a more reddish hue ; the tibiæ without the lanceolate scales, metatarsi slightly but distinctly shorter than following joint ; abdomen slender, very pilose, black, slightly longer than wings. The most prominent divergence from female is in the shape and size of the joints of the antennæ ; first joint is much larger than the corresponding joint of the female ; the second somewhat larger but of the same shape, each joint from the third to the tenth spheroidal, becoming

more and more distorted and oblique to the tenth, which is of an irregular oblong shape. Joint 11 is much elongated with the plumosity inserted on an oblique line near base; joints 12–14 much shorter than 11, but longer than any of the basal ten joints. Joints 2, 12 and 13 with a circlet of bristles in place of plumosity; joint 1 naked, 14 without bristles or plumosity but pilose, as are joints 11, 12, and 13 in addition to the plumosity and bristles; joints 3–11 inclusive plumose with a narrow white circlet in front of the insertion of plumosity. Antennary sense organs smaller and straighter than those of the female. Neuration of the male wing practically as in female except that the first subcostal cell is wanting; its place being occupied by the fusion line of the auxiliary and cubital veins; anal veins stronger and more distinct than in female.

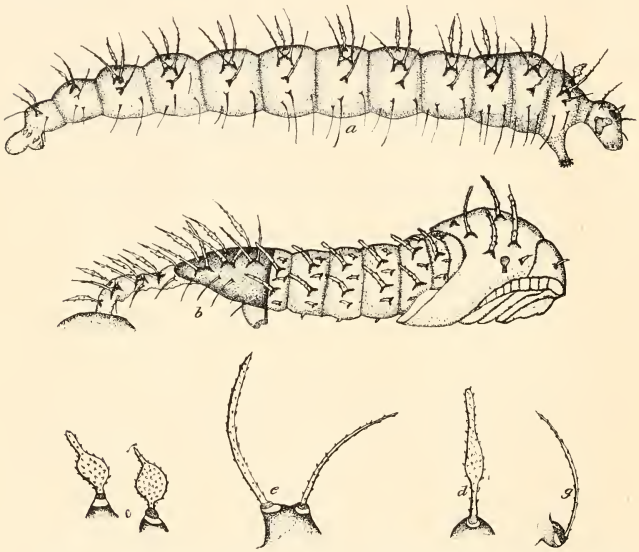


FIG. 3. *C. specularis.* *a*, larva; *b*, pupa; *c*, pair of prothoracic bristles; *d*, dorsal bristle on remaining segments; *e*, furcate tubercle with its two bristles; *g*, small lateral bristle.

Larva: 4–5 mm. long, cylindrical, color dirty white, brownish at the insertion of bristles; head brown, eye spot and mouth parts black. Thoracic legs, .3 mm. long, cylindrical, white, armed at tips with a circlet of black claws; anal feet with two transverse rows of black claws. Chætotaxy for lateral half of head and body as follows: Head with two ovate-lanceolate hyaline bristles, one in front of, the other behind the

typical head-spine or horn, in addition to several scattered simple bristles. Body minutely pubescent; ovate lanceolate bristles in one dorsal longitudinal row; thoracic pair not enlarged; dorsal bristles smooth, hyaline, bulbous at base. The insertion of the pair on each segment often connected by a transverse black line; 3 longitudinal lateral rows of barbellate bristles inserted below the ovate-lanceolate ones; bristles black, shining, with short and smooth bristles near the ventral surface, the last two segments having only the upper row of these barbellate bristles.

Pupa: 3 mm. long, spinous, light yellow; head darker, obtuse, notched below; thorax with three spines on either side, spines barbellate, anterior one mucronate; stigma spatulate; abdominal spines short, rough, mucronate, in five longitudinal rows on either side; those of rows 1 and 4 larger and longer than the others; there is also a ventral row of very short thick spines. Pupa attached to under surface of dung by the old larval skin.

During November, December and January the larvæ of this species were found in immense numbers on the under side of nearly dry cow dung. They seem to feed on the dung, never penetrating very far into the substance. No eggs were found. The duration of the larval stage seems to be several weeks, that o the pupal stage 7–10 days.

The sense organs on the antennæ of the imagines can be seen only with a high power (500 dia.) and then it is necessary to focus carefully to find them, but once found, they stand out plainly. None of the wings of the males had two subcostal cells and many wings of the females had only one; but in some wings the two cells show plainly. Several hundred larvæ of all ages were found on the under surface of a piece of moist rotting elm wood; similar larvæ and puparia were also found in the nests of the common foraging ant (*Eciton cæcum*) on several different occasions; the author bred imagines from the larvæ taken in these various habitats, and they proved to be the same species. This seems to be a strictly winter species, none of the various stages being found during the warmer months.

Ceratopogon specularis Coquillett.

♂, length 2.5 mm., wing 1.5 x .45 mm.; ♀, length 2 mm., wing 1.5 x .6 mm. One subcostal cell, third or cubital vein terminating distinctly before the middle of the wing; with a faint white spot at apex of costa vein; wings and entire insect densely pilose; pile black; halteres white; metatarsi equalling or slightly longer than following joint; antennæ not

longer than mesonotum ; tibiæ of the female without lanceolate scales ; last four joints of male antennæ longer than basal ten.

♀, head black, both extremities of each segment of the palpi whitish, especially the anterior extremities ; central and major portion of each segment black ; proboscis with three narrow transverse whitish bands near apex. Pile on antennæ black, whitish at tips ; antennæ reaching to about the middle of the mesonotum ; joints globose, subequal, the last four being larger and more oblong, especially the apical one, which is prolonged into a short blunt point ; basal joint much larger and more globose than the others ; sense organs, insertion and position of the pile and bristles on the antennæ as in *C. brumalis ;* mesonotum black, shining, pile black ; sides of prothorax with a bunch of black bristles ; pleuræ naked ; mesopleuræ whitish ; abdomen black, shining ; pile on posterior anal segments dirty white, longer than on other parts, central portion of each segment of venter black ; legs black ; tarsi distinctly yellow, infuscated at tips, each joint with a graduated row of bristles on the inner plantar surface ; empodium

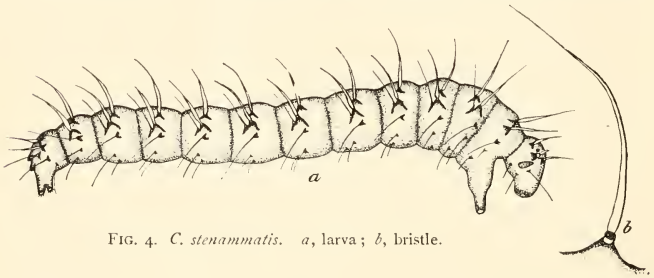


FIG. 4. *C. stenammatis*. *a*, larva ; *b*, bristle.

strap-shaped, curved, bristly-fringed on convex side ; neuration of wings differing from that of *C. brumalis* in the following points : subcostal cell single ; only one anal vein ; no obsolescent veins in cubital or discal cells ; anterior costal cell very narrow ; juncture of the veins at base of wings more distinct.

♂ Coloration practically as in female, pile on entire insect longer, denser and more erect than on female ; the basal joint of antennæ as usual much enlarged, globose ; joints 2–10 inclusive subglobose, graduating in size toward apex and becoming somewhat oblique ; joints 11 and 12 much elongated, subequal, tapering somewhat toward apex ; joint 13 ovate-oblong, smaller and shorter than the apical one. Plumosity, pilosity and sense organs of antennæ as in *C. brumalis ;* also neuration of wings as in female.

Larva : 5–6 mm. long, bristly, and minutely black spinulose ; cylindrical, tapering slightly from the metathoracic segment toward base, tapering rapidly toward the head ; dirty white. Head dark brown, eye spot and mouth parts black ; tips of thoracic feet armed with a circlet

of black claws; anal feet fleshy, armed with two rows of black claws. Chætotaxy of lateral half of head and body as follows: head ornamented behind with one honey yellow, semitransparent, barbellate bristle, in front of which is a large short simple spine, with white bulbous base, in addition to a few simple bristles. Body with three longitudinal rows of barbellate bristles, dorsal bristles honey yellow, enlarged in center; thoracic pair much enlarged and shorter than the others; the dorso-lateral bristles rise from double tubercles, which are simple on the last two segments; anterior limbs of these tubercles tipped with long honey yellow bristles; those of the posterior limbs short and black; lateral row of small, simple tubercles tipped with dark brown bristles; then subventrally are several scattered simple bristles.

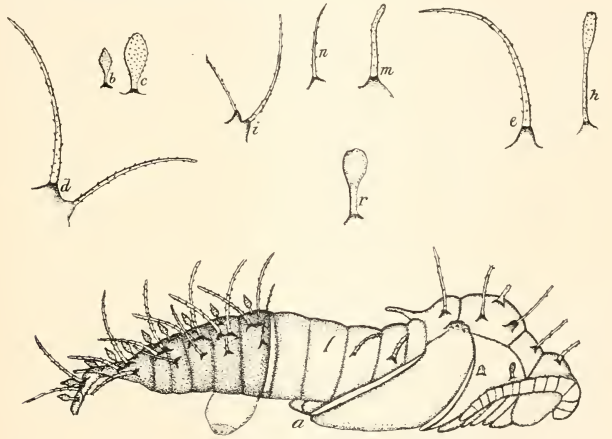


FIG. 5. *C. wheeleri*. *a*, pupa; *b*, dorsal bristle; *c*, enlarged prothoracic dorsal bristle; *d*, furcate tubercle with its two bristles; *e*, lateral bristle.

C. texanus. *h*, dorsal clavate bristle; *i*, lateral furcate tubercle with its bristles; *n*, small lateral bristle; *m*, characteristic spine of pupa; *r*, thoracic stigma of pupa.

Pupa: 2.5–3 mm. long, pale yellow, head darker. Thorax with four large barbellate spines on each side and several small ones with mucronate tips; anterior abdominal segments which are not incased in larval skin, with five longitudinal rows of barbellate spines; the spines of rows 2 and 4 longer and larger than the others. Ventrally is a row of very short spines with mucronate tips. Anal segment forked with two finger-like bodies at base of furcation. These tips were divergent in life and probably serve to retain the larval skin on the anal segments as the spines are obsolete under this skin. These appendages were observed in all the species of which puparia were seen. Duration of pupal stage 8–10 days.

The larvæ are gregarious and live on the under side of dry cow dung from August to December, but are more abundant during November and December, though not so common as *C. brumalis*. The larvæ of both species are sometimes found under the same piece of dung but usually each species is found alone. This species is described here at length as Mr. Coquilett has given only a brief description of the male.[1]

CERATOPOGON STENAMMATIS, sp. nov.

Larva : 5 mm. long, anal segments with two cylindrical feet, tips armed with black claws ; feet .15 mm. long ; thoracic legs .45 mm. long ; color dirty white ; head and mouth parts dark brown. Head with two smooth, short, slightly enlarged bristles, one anterior and the other posterior, to the typical head spine, besides a few scattered simple bristles. Either side of body with two longitudinal rows of elongated setigerous tubercles, the dorsal row with only one hyaline acerose bristle to each tubercle, the lateral row with two bristles to each tubercle, one from apex similar to dorsal bristle, the other simple and of a brownish color and from the posterior side of the tubercle ; there are besides several scattered simple bristles along the ventral and subventral surface.

The specimens were received from Dr. W. M. Wheeler, who found them in the nest of an ant (*Stenamma fulvum* subsp. *aquia*) at Colebrook, Conn., August, 1900. They were moving about in the refuse heaped up by the ants in certain portions of their nests. The species seems to be a genuine myrmecophile like the European species (*C. Braueri* Wasmann).[2]

CERATOPOGON TEXANUS, sp. nov.

♂, length 2.5 mm. ; wing, 1.3 x .4 mm. ; ♀ length 2 mm., wing 1.35 x .5 mm. ; wings and entire insect densely pilose ; one subcostal cell, third or cubital vein terminating distinctly before the middle of the wing ; no white spot at apex of costal vein ; halteres white ; metatarsi slightly longer than succeeding joint ; tibia of the female without lanceolate scales.

♀, head dark brown, pile brown ; joint 1 of antennæ large globose ; joints 2–10 globose, graduating to globose-ovate, subequal ; joints 11–14 ovate-oblong, apical one larger. Sense organs and arrangement of pile as in *C. brumalis*. Second joint of palpus in female much enlarged. (See Fig. 6.) Mesonotum drab, with short, appressed, light yellow pile, intermixed with some stouter black hairs ; scutellum drab, with several rows of long black hairs ; sides of prothorax with a bunch of hairs ; pleuræ naked,

[1] Vide, "Some New Diptera," *Proceedings of the National Museum*, Vol. XXIII., 1901, p. 601.

[2] Vide, "Eine myrmecophile Ceratopogen-Larve," *Wiener Entomologische Zeitung*, XII. Jahrg., 8. Heft (10 October, 1893) pp. 277–279.

dirty white, abdominal segments dark brown with dirty white edges producing a banded appearance, which becomes obsolete on the last segments ; pile short, appressed, grayish, becoming longer toward anal segments ; venter dirty white, densely pilose on sides, each segment with long dark pile in front, becoming light yellow behind ; legs dark, pile yellowish and of varying lengths ; tibia of hind legs with a circlet of short spines at apex ; first joint of tarsus slightly shorter than second ; empodium and neuration of wings as in *C. specularis.*

Coloration of male darker throughout than in female ; antennal joints 11–14 inclusive equalling in length the remaining joints ; first joint globose, enlarged ; joints 2–10 depressed globose, graduating in size toward apex and becoming somewhat oblique ; joint 11 much elongated, nearly twice the length of any succeeding joint ; joints 12–14 subequal, apical one slightly larger and more oval-oblong. Pile, plumosity, sense organs, and neuration of wings as in *C. specularis.* Last four segments of abdomen without the whitish edges, blackish, shining, pile denser and longer, without the peculiar pile on venter.

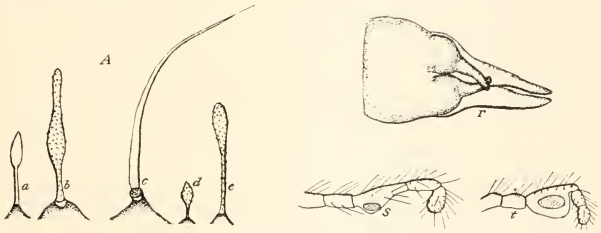


FIG. 6. *A*, characteristic dorsal bristles of each of the five species above described ; *r*, anal segment of the pupa of *C. specularis ; s*, palpus of female of *C. brumalis ; t*, palpus of female of *C. texanus.*

Larva : Length 5 to 5.5 mm. ; dirty white ; head with two semihyaline bristles ; one in front and the other behind the typical head spine ; chætotaxy for lateral half of body as follows : three longitudinal rows of barbellate bristles ; dorsal bristles hyaline, clavate, minutely tuberculate with segments 5–10 joined at base by a straight black line ; dorso-lateral row of double tubercles, the anterior limbs of which are tipped with curved, semihyaline, broad bristles ; those on posterior limbs short and black ; lateral row of bristles sparsely barbellate, black at base graduating to hyaline at apex ; under the above there are several smaller and subventrally placed simple bristles on each segment ; anal segment with an extra pair of dorsal bristles behind the regular ones. Legs as in *C. brumalis.*

Pupa : 2.5 mm. long, whitish translucent, thorax armed on either side with five yellowish hyaline barbellate spines, the frontal one small with mucronate tip just above and near the base of the antenna. The others on the thoracic dorsum at the corners of a rhomboid ; the anterior spine

truncate with mucronate tip. Stigma black, peduncle semihyaline, the four abdominal segments anterior to the larval skin with three longitudinal rows of spines; those of the dorsal and lateral rows large, hyaline, curved and barbellate, and rising from very prominent tubercles; subvental row of somewhat smaller spines; the tubercles point forward while the spines curve outward and backward. Duration of pupal stage: 7 to 8 days.

The larvæ of this species are gregarious in small numbers beneath the bark of old dead trees in moist places, or on the under side of very damp rotting wood, during December and January. Rare.

CERATOPOGON WHEELERI, sp. nov.

Larva: 5–6 mm. in length; chætotaxy for lateral half of head and body as follows: head with two brown barbellate bristles, one in front, the other behind the typical head spine; besides several scattered simple

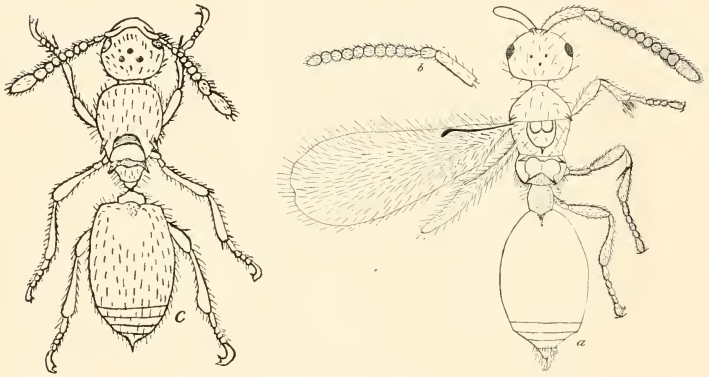


FIG. 7. *a*, ♀ of *Adeliopria longii*; *b*, ♂ antenna; *c*, ♀ of *Solenopsia imitatrix* (redrawn from Wasmann).

bristles; body with three longitudinal rows of barbellate bristles; dorsal row of bristles minutely tuberculate, globose-ovate, hyaline; prothoracic pair much enlarged and more globose; dorso-lateral bristles rise from double tubercles, those on anterior limbs long, light brown and semi-transparent, posterior branches with shorter, jet black bristles; lateral row of small simple tubercles tipped with black shining bristles.

Pupa: 2.5–3 mm. long, head and thorax wine-colored; either side of the thorax with six barbellate spines of varying length and one large tubercle-like spine near stigma, thorax heavily sculptured and minutely spinulose; segments 2 and 3 with a barbellate spine on either side; segment 4 with one small simple spine; other segments unarmed, shining, with small irregular ridges on dorsal portion; anal segment encased in larval skin.

Each of the puparia had been killed by an interesting prototrypid parasite so that no imagines of this new myrmecophilous *Ceratopogon* could be obtained. A number of these parasites were sent to Mr. Ashmead for identification and proved to represent a new genus of the family Diapriidæ. Through the kindness of Mr. Ashmead, his description of this new genus and species is inserted in this article. By way of illustration figures are included of this new species (*Adeliopria longii* Ashmead) and also of the new and closely related species (*Solenopsia imitatrix*) recently described by Wasmann.[1] The Ceratopogon puparia were found December 15, 1900, beneath a stone, in what seemed to be an abandoned ants' nest. The parasites issued, one from the thoracic dorsum of each of the *Ceratopogon* puparia December 31 and lived eight or ten days. This suggests that some of the proctotrupids that have been found in ants' nests and regarded as myrmecophiles may be in reality only parasites of the guests of the ants.

A comparison of the five species above described shows that the larvæ of each have practically the same general size, shape, and color; but the spinous ornamentation is very different, so that they are readily separable by this means alone. In *C. stenammatis* the larvæ differ from the other species in the shape of the anal feet which are cylindrical, short and armed with claws at apex.

The puparia of the Texan Ceratopogons also resemble one another very much. Even the differences in spinous ornamentation are not so marked as in the larvæ. The imagines, however, differ in many essential details, especially in coloration and pilosity. The palpi of both sexes of *C. brumalis* and *C. specularis* are similar, but the female of *C. texanus* has the second joint of the palpus much enlarged and the sense organ on it correspondingly increased in size (see Fig. 6, *s* and *t*); the neuration of the wings of the species is practically identical; the anal extremities of all the puparia which were examined by the writer have the peculiar furcate anal tip and "fingers" described under *C. specularis* (see Fig. 6, *r*). These probably function, as suggested, in holding the old larval skin to the posterior segments of the pupa.

[1] Die psychischen Fähigkeiten der Ameisen, 1899, p. 127 and taf. III., figure 1.

This seems probable also from the fact that the segments encased in the larval skin are smooth, with none of the spines or bristles which appear on the free portions. The tips were observed to be divergent in life but the exact position of the "fingers" could not be determined on account of their being concealed under the larval skin. The dorsal bristles of each of the five species (see Fig. 6, *a*) are the most characteristic and distinguishing features of the larvæ, thus affording a sure and easy means of separating the species. In no case is there any material variation in these bristles for a given species; even when the habitat varies considerably, as in *C. brumalis*, which lives under dung, under rotten wood and in the nests of ants (*Eciton cæcum*), the spinous ornamentation is constant, and the imagines are identical.

A NEW GENUS OF DIAPRIIDS FROM TEXAS.

WILLIAM H. ASHMEAD.

Recently I received from Mr. W. H. Long, Jr., of Austin, Texas, several specimens of a Diapriid, bred by him from puparia of a Dipteron, *Ceratopogon* sp., obtained from an abandoned ant's nest under a stone.

A careful examination of the specimens shows that both sexes are represented and that they will form the type of a new genus allied to the recently established genus *Solenopsia* Wasmann ;[1] but quite distinct in several particulars. At the request of Mr. Long, it is here described to be incorporated by him in a paper that will appear shortly.

Adeliopria, gen. nov. (Diapriidæ).

The two genera may separated as follows :

Females..... 2
Males..... 3
2. Antennæ 11-jointed, the funicle joints 2–7 transverse, the 8th joint quadrate, the club large cone-shaped, unjointed.....*Solenopsia* Wasm.
Antennæ 12-jointed, the flagellum clavate, the funicle joints 6–9 moniliform, the club fusiform, 3-jointed.....*Adeliopria*, n. g.
3. Antennæ 12-jointed ; mesonotum without parapsidal furrows.
Flagellum ending in a 3-jointed club.....*Solenopsia* Wasm.
Flagellum filiform, sparsely, finely pubescent, tapering off at tip, joints 2–11 moniliform, the last joint small, oval.....*Adeliopria*, n. g.

Adeliopria longii, sp. nov.

♀ ♂. Length, .8 to 1 mm. Polished, black, impunctate, the metapleuræ clothed with a pale pubescence ; the head and thorax with a few fine long hairs ; the head transverse, the temples obliquely rounded off posteriorly ; scape, mandibles and legs rufo-testaceous ; the legs in ♀ a little the darker with the sutures of trochanters, a dot on knees and the extreme apices of tibiæ yellowish ; in ♂ with the front and middle coxæ yellowish, the scutellum has a large fovea across the base, separated into two by a delicate median carina at base. Metathorax wrinkled with a slight median spine at base, just back of scutellum. Wings hyaline, subemarginate at apex, the apices being cordate ; the venation as in *Phænopria* and ciliate. Abdomen oblong, smooth and polished, the second segment very large, occupying most of the surface ; the petiole longer than thick, furrowed. Type :—Cat. No. 5715, U. S. N. M. (8 ♀ and 2 ♂ specimens) Host. Dipt.; *Ceratopogon wheeleri* Long.

[1] Vide Die psychischen Fähigkeiten der Ameisen, 1899, p. 127.

TWO NEW EMBIIDÆ.[1]

AXEL LEONARD MELANDER.

The singular and primitive family Embidæ of doubtful affinities is represented by not more than a score of living species and a few preserved in amber. Of these nearly one half are known from fewer than five specimens each. Up to 1885, the date of publication of Hagen's Monograph of this family,[2] only fifteen living species had been recorded. Since that time less than a half dozen species have been discovered, so that even now this family remains one of the most poorly represented of the insect world.

The habits of three or four species have been studied to some extent, but only of *Embia solieri* Rambur do we know anything at all approaching the whole postembryonic life-history. It is to Professor Grassi[3] that we owe this contribution. He worked out the internal anatomy of this species and showed how the nests in which it lives are spun by the insect's forefeet instead of by its mouth-parts as had been previously supposed. The object of the present paper is to call attention to two new forms, one from Texas and one from Mexico, and to the peculiar structure of the spinning organ.

Up to the present time only two species have been taken on this continent, one, *Embia* (*Oligotoma*) *hubbardi* Hagen, in Florida and one, *Olyntha salvini* McLachan in Mexico. The new forms probably belong to these same genera, though one of them is here placed in *Embia*. This is done, not because it ought not to be included in *Oligotoma*, where its nearest allies are placed, but because it is believed that the genus *Oligotoma* is untenable. The two genera are separated only by a peculiarity of wing neuration, but Grassi has shown that the adults of

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 16.

[2] Hagen, H., Monograph of the Embidina, *Canadian Entomologist*, Vol. XVII.,, pp. 141–155, 171–178, 190–199, 206–229.

[3] Costituzione e Svillupo della Societa die Termitidi . . . con un' Appendice . . . sulla Famiglia delle Embidini. Catania, 1893.

Embia solieri are wingless, so that in this case the character can not apply. None of the specimens of either of the new species show any traces of wings, although those of the Texan species which were sectioned possess spermatozoa nearly matured. It is interesting to note the distribution of the North American genera. The two *Olyntlias* were taken on nearly the same parallel of latitude, and this is also the case with the two *Embias.*

The types of the two new species are deposited in the Museum of Comparative Zoölogy, which also contains the material worked over by Dr. Hagen.

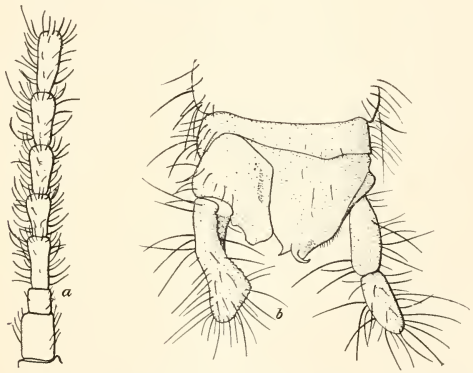


FIG. 1. *Olyntha wheeleri*, sp. nov. *a*, base of antenna; *b*, dorsal view of tip of abdomen.

OLYNTHA WHEELERI, sp. nov. (Fig. 1.)

Length, 6.5 mm. Body black, very lightly subpruinose, punctulate, subglabrous. Insect sparsely covered with fine pale hairs and slender black bristles scattered on antennæ, above eyes, on femora, dense on under surface of four posterior metatarsi, more sparsely on lateral edges of thorax and abdomen, and long on anal appendages. Antennæ somewhat defective, but with twenty joints present, which together are longer than head and thorax; basal joint stout, cylindrical, second one fourth less in diameter, its length and diameter equal, first and second joints piceous, with no erect black bristles, remaining joints dark fuscous with black radiating bristles, third antennal joint equal to two basals, its sides concave, remaining joints elongate pyriform, thicker beyond middle, fourth shorter, fifth to sixteenth subequal, seventeenth to twentieth a little shorter. Labial palpi rather

thick. Maxillary palpi plainly five-jointed, second and third joints subequal, each a little shorter than first, fourth longer than first but more slender, fifth not longer than third plus the fourth, with numerous pale hairs. The bases of the maxillæ and the galeæ are testaceous, remainder of head black. Thorax flattened above, the mesothorax larger than the others, evenly black, no color markings. Legs black, last tarsal joint alutaceous at tip, ungues pale with piceous apex; fore metatarsi greatly enlarged, with pale recurved hairs along edge of lower surface, middle joint with similar hairs: tarsi of four posterior legs slender, metatarsi with a dense brush of thick, short, black bristles beneath, middle joint with paler papilla, third joint slender, long; middle legs slender; posterior femora much incrassated. Left anal appendage one-jointed, but articulated with the body, stout, large, clavate, obliquely truncate at tip, lightly rugose. Right appendage two-jointed as usual, the apical joint shorter and thinner than the basal. Both appendages have many long brown to black hairs. Between the appendages are two triangular lamellæ. The left one testaceous, the right one black and testaceous apically: each armed with an apical spine pointing transversely outward. The median portion of the last ventral segment is placed largely towards the right. From the upper surface the secondary sexual characters present a different aspect. The terminal abdominal segment (10th) is obliquely cleft, the cleavage starting near the left side and terminating nearly midway between the appendages. The left portion is prolonged into a hastate slender projection. Near the base of the left appendage arises a flat lighter-colored triangular process (seen from below as the left triangular lamella). This overlies the hastate projection. The right portion of the last segment terminates in a flattened bifurcate process, the outer tooth of which is bent downward and the upper tooth to the right. The ninth abdominal dorsal segment is very narrow, of about one third the depth of the others: its posterior margin is arcuate, bulging outward on the right side and emarginate on the left.

From above the head is one half longer than broad, wide at the rather prominent eyes and then sloping suddenly forward and less so behind the eyes; hind angles very oblique; head not at all quadrangular in outline; no impressed markings; surface evenly, finely rugulose.

Among other characters, the shape of the head, the structure of the antennæ, the color of the hairs and body and the remarkable sexual organs are peculiar to this species and readily distinguish it from the other species of *Olyntha*.

One wingless specimen from Cuernavaca, Mexico, taken December 26, 1900, by Dr. Wm. W. Wheeler while excavating a nest of *Leptogenys wheeleri* Forel.

EMBIA TEXANA, sp. nov. (Figs. 2 and 3.)

Immature Male.—Length, 5–7 mm. Rufous. Antennæ pale fuscous, when folded back reaching to second pair of legs, filiform with small testaceous setæ. First three joints more or less quadrate, others rounded, first joint rather thicker than the others, a little longer than broad: second the palest, as long as broad: third pale at base, nearly twice as long as broad: fourth and fifth but little longer than broad: remaining joints gradually increasing in length, last three joints twice as long as wide, their diameter nearly equal to that at base. No more than sixteen joints were

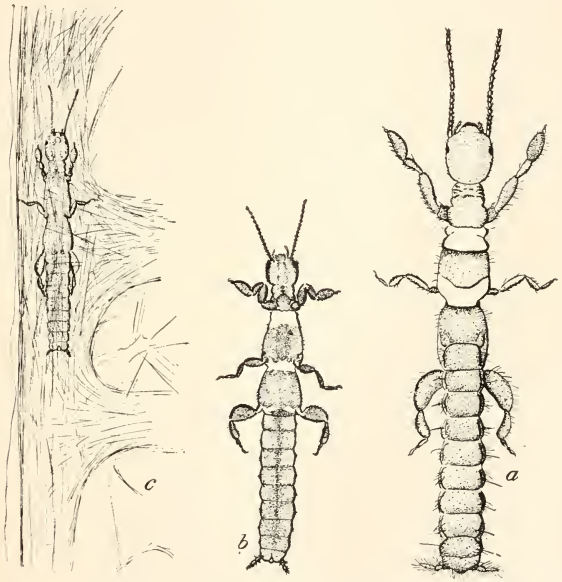


FIG. 2. *Embia texana*, sp. nov. *a*, dorsal view; *b*, ventral view; *c*, insect in web.

counted in any one specimen. Each antennal joint has a small pale space at its base. The 5-jointed maxillary palpi become paler apically and have the first joint connate with the head, second joint very short, third, fourth and fifth gradually increasing in length and decreasing in breadth, fifth a little longer than the two preceding together. Third joint of the labial palpi broad, yellow. Galeæ yellow, prominent, broad. Mentum and submentum rufous, as is a large W-shaped mark on the lower side of the head. Eyes oblique, black, consisting of about fifty facets. Neck tri-

angular beneath, with two small rufous tubercles, between which are the apices of two rufous triangles. The thoracic sclerites transverse, rufous, separated by colorless thin chitin. The sterna are well-marked. Abdominal sclerites more firmly chitinized than thorax and therefore darker red. Anal plate divided, a little asymmetrically, the right piece with a slight subapical notch on inside where there is a transverse carina. If any differences exist in the anal styles the basal joint of the left is a little stouter. Legs rufous except middle and hind coxæ and trochanters, knees, and last tarsal joint. The fore and hind femora are strongly incrassate as are the fore tibiæ and tarsi. Under side of the front metatarsi nearly plane, beset with minute and with moderately long bristles, the longer set recurved at tip. Remainder of legs and body covered with scattered pale yellow

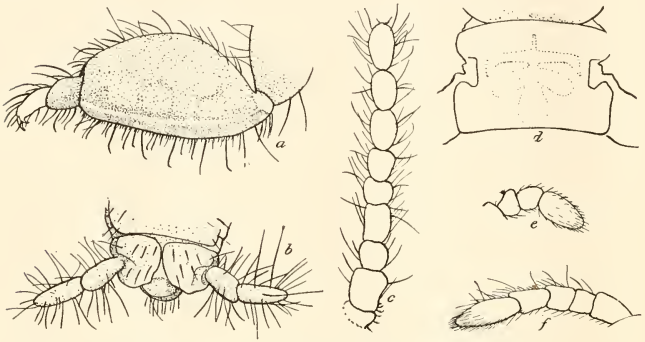


FIG. 3. *Embia texana*. *a*, front tarsus; *b*, tip of dorsum of abdomen; *c*, base of antenna; *d*, prothoracic notum; *e*, labial palpus; *f*, maxillary palpus.

bristles which are longer on the anal styles. Upper side of head with three clear "ocellar" spots, a Y-shaped mark confluent with an inverted heart-shaped, basal space, on each side of which is a granulate clavate mark. The stem of the Y continues along the middorsal line of the thorax and more faintly along the basal segments of the abdomen. Upper side of the thorax with an inverted "fleur-de-lis"-shaped, granulate, clearer mark. These color-marks are sometimes faint. No traces of wings.

Eight specimens taken during the winter months, from November to March. Austin, Texas.

The following are the chief characters which separate the related species from the above-described form:

Embia (*Oligotoma*) *cubana* Hagen. *Winged male.* Head cut straight near the prothorax; last palpal joint long-conical; between anal styles above is a short bent process, on the left side

of which is a conical lobe, short, open at tip. *Wingless female.* Antennæ 18-jointed; last ventral segment not divided; above between appendages is a small thin elongated lobe.

Embia (*Oligotoma*) *hubbardi* Hag. *Winged male.* 4 mm. Head cut straight before prothorax; second joint of antennæ very small, annular; third joint as long as two basals, thicker on tip; fourth and fifth similar to third but a little shorter; abdomen pale brown.

Embia (*Oligotoma*) *insularis* McLachl. *Winged male.* Sixth antennal joint as long as third; apical joint of labial palpi ovoid; abdomen pale dirty brown; last ventral ending in a bottle-shaped tube which is turned to the left and partly surrounded by a horny hook.

Embia texana is very susceptible to differing degrees of humidity and therefore cannot be found at all seasons of the year. After the ground has been moistened by the winter rains the Embiids were found, the last one being taken during March. As soon as the overhead sun dries up the ground these insects are never seen, having burrowed deeply into the soil. One specimen was taken while ripping the bark from a fallen tree, all the others were found under stones; and of these one was found in company with *Formica fusca* L. var. *gnava* Buckley, though its association with the ants was accidental, no doubt, as was shown by its hurry to escape. In this connection attention may be called to the occurrence of the *Olyntha* in the nest of *Leptogenys*. *Embia texana* is apparently not rare in this vicinity, two having been taken in a single day, though from its neat manner of concealment and its limited time of appearance it is found only by the merest chance. The species has been taken in localities about the city of Austin separated by over ten miles, places of different character, one a sandy tract, and the other a limestone hill.

The insects live singly in silken webs, spun by themselves. These webs are tunnels in some niche of the rock which shelters them, or spun between the grains of soil. They are an inch or more in length, and closed at one end, their diameter a little greater than the insect's body. Sometimes a small flat web is spun beneath the stone. The tunnels are provided with side-

passages ending, in the case under observation, blindly (Fig. 2, *c*). When the Embiid wishes to turn in its passage it backs partway into a side tunnel until its head is free to point the other way. This is not universal, however. *Embia* was several times seen to turn on itself, as its very supple body and unchitinized joints permit it to place the thorax in a line parallel with and touching its abdomen. During the daytime *Embia* seemed loath to quit its tunnel, and when forced out would always return to it in a few minutes. When in the tunnel it is completely concealed. This accounts for the few specimens, as all were taken when driven from their retreat on the removal of their stone.

It was stated by Hagen that the web is used in ensnaring prey. Its texture is far too delicate for that purpose, even were its size larger. *Embia's* silk is very different from spiders' webs. It is much more frail and of an opalescent whiteness which renders it distinguishable, after practice, from the spider webs common in the same situations. Grassi states: "Evidently the gallery serves to protect the body from too excessive transpiration, and to keep about the *Embia* an atmosphere not too dry," but it is difficult to see how a net can prevent excessive transpiration in a dry climate like that of Austin. The tunnels probably serve merely as a retreat. The insect seems entirely contented when at home and touching the meshes of its web, so that it may be described as strongly, positively thigmotactic. Indeed, it was frequently observed to stretch its front feet outward, in order to press its back against the soft silk.

When the web was touched, the Embiid darted out, sometimes head first and sometimes crawfishing. Its backward movements, however, are very different from its normal walk. In moving ahead *Embia* walks with a sinuous motion, bending its supple body slightly to accommodate the motions of its legs, and covering about two thirds of an inch, or less, in one second. Its recoils are more of a scurry from danger. It then travels a full inch in a second and in a straight path, though it never goes beyond an inch in any single dart. While walking, the abdomen is carried in a very unstaphylinid-like manner, the central part being elevated and the tip depressed. *Embia* was never observed to jump, though the incrassate and muscular femora would at first sight seem to indicate that habit.

One of the *Embias* was kept in a small culture dish with a few grains of sand. The first night it spun along the edge of the glass a web in which it remained the two following days. Then it discovered the sand in the center of its room and spun another gallery between the surface of the glass and the sand. In this position it was absolutely concealed, having completely covered the web with the small amount of sand present. *Embia* is exclusively nocturnal, at least in the nymphal state. Although the tunnel was in great part completed in a single night additions were made to it on several succeeding nights.

At first sight the silk resembles a very thin, pale gossamer, but when viewed under a strong magnification the individual threads are seen to be arranged in fine bundles of varying thickness. Most of the threads in a bundle lie parallel with one another, but the bundles contain a few curled threads also. The individual threads vary in diameter.

In making its web *Embia* does not use its mouth—notwithstanding Hagen's statement that spinning organs are present among the mouth parts—but it uses instead, its forefeet, as Grassi had shown. No other animal has developed spinning organs in this position. The silk is produced by large glands occupying nearly the whole of the enlarged metatarsus. The underside of this joint is provided with a dense mat of small short bristles, and interspersed among them are the longer spinning bristles, each of which is hollow and slightly recurved at the tip and contains a tenuous duct from the gland. While spinning, *Embia* very rapidly moves its forefeet, either singly or both together, now reaching out directly in front, now to the side, with a movement which reminds one of a cat toying with a string. Each time it touches a surface it attaches a bundle of silk. As the bundles are of varying sizes it would seem that sometimes all the glands function, at others only one.

While spinning its cylindrical retreat, *Embia* slowly rotates on its longitudinal axis. When within its nest it does not find it inconvenient to rest with head downward. It was seen resting on its side or back more frequently than with its ventral surface down. The soothing touch of its web seems to be suffi-

cient to insure a feeling of security no matter how its body may be placed. In its natural habitat the insect is normally found clinging to the under surface of its sheltering stone. But when driven from its gallery, *Embia* rebels if placed in an inverted position and rights itself immediately by wriggling the tail and clutching with the forefoot, which probably at the same moment emits a strand of silk.

The antennæ of *Embia* appear to be less sensitive to touch than the body. The insect would frequently butt against some obstruction notwithstanding the warning received through the antennæ. On one occasion the warning was insufficient to prevent *Embia* from walking into a drop of honey, and after an enforced bath the insect seemed more solicitous of its forefeet than of the rest of its body, carefully eating off the honey from their plantar surfaces. When about to clean its antennæ *Embia* rapidly turned its head and thus brought one of these organs under the body and then standing over it gradually drew it forward, at the same time eating off the honey. This was the only time the insect was observed to eat anything of a vegetable origin, and then it was only with great reluctance. On several occasions small pieces of a worm and a fly attracted its attention but after a few nibbles it would run away. The abandoned web at the edge of the jar seemed to be a more toothsome morsel, for *Embia* was frequently observed nibbling at it.

The spinning glands of *Embia* (Fig. 4) are unique and without parallel. The silk is produced in the thickened anterior metatarsi within chambers and conducted outside to the recurved hairs at the edge of the plantar surface. The chambers of the metatarsus which may be seen shining through the chitinous integument, appear more or less regularly arranged in three longitudinal layers, one next to the sole, a second in the middle, and a third dorsally. In each series there are, roughly estimated, about twenty-five chambers, placed about three abreast. Thus in the whole joint the chambers number between seventy-five and eighty. The cavities are nearly all of the same size—about sixty micra in diameter—though the outer chambers are somewhat smaller. Each chamber is more or less cuboidal and bounded by a single layer of epithelium. This is for the most part quite

thin and flattened in the central part of the faces, but becomes columnar at the corners. Cell-boundaries are not easily seen. The large central space of each chamber is filled with a colloid secretion, more or less shrunken in alcoholic material. This substance, the silk, is carried to the tips of the hairs through ducts of varying length, one from each chamber (Fig. 4, *b*). The terminal hairs (Fig. 4, *a*) are arranged in a row around the edge of the plantar surface of both the metatarsus and the second tarsal joint. Though the latter is devoid of any secreting gland it possesses several ducts leading through it from the metatarsus. Some of the ducts lead along the periphery of the joint, others

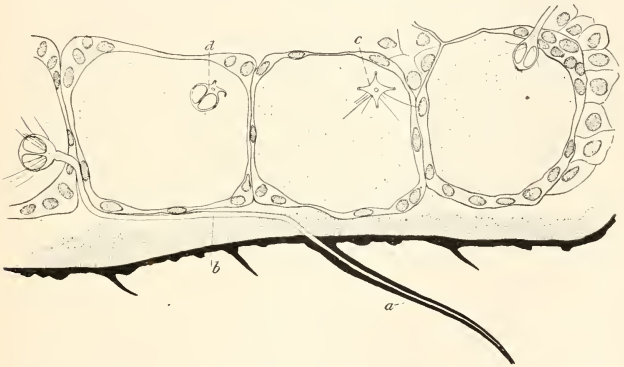


FIG. 4. *Embia texana*. Portion of metatarsus, showing glands; *a*, spinning bristle; *b*, duct of silk-tube; *c*, ampulla at base of duct.

pass between the gland-chambers. The foot-tendon lies in large part between the two lower series of glands.

In the glands, each duct arises from a remarkable ampulla (Fig. 4, *c*, *d*, *e*). The wall of the duct suddenly becomes thickened, and then subdivides into four or five rays, which are continued in the form of the equidistant meridians of a sphere and meet again in an end-plate opposite the point of subdivision. Thus the lumen of each duct terminates proximally in four, or rarely five, large, elliptical or ovoid, lateral openings. At the base of each opening is a radial arrangement of fine processes, possibly the expression of the silk being drawn into the lumen of the tube.

UNIVERSITY OF TEXAS, AUSTIN, TEXAS, March 22, 1901.

Postscript.

Since the foregoing account was written we have met with *Embia texana* a number of times, while on two occasions several specimens were associated in the same nest. In the former of these cases the retreat was the combined efforts of six individuals, in the latter but three specimens resided together. These family homes are larger than those of the solitary *Embias*, extending for several inches and consisting of a large mass of silk. As none of the specimens observed show any trace of wing-formation, our previous conclusion, that this species is wingless, is probably correct.

Austin, Texas, March 22, 1902.

THE NESTING HABITS OF ANTHIDIUM.[1]

AXEL LEONARD MELANDER.

The care and forethought which an insect shows in providing for an offspring which in most cases she will never see have always excited the interest of biologists. Among these insects the solitary bees have especially attracted the attention of observers on account of their varied habits of nest construction. Here, in a group of insects more or less homogeneous, we find the style of architecture under such differing forms as the fence-rail excavations of the carpenter-bees, the thimble-like nest of the leaf-cutters, or the *Andrena*-burrows in wayside paths.

The genus *Anthidium* affords no less interesting methods of house construction. For many years the habits of a few species of this genus have been well known, but they constitute but a small contribution when the whole number of species is considered. In a recent monograph of the European species of *Anthidium* Dr. Henry Friese estimates the total number of species of the world at between five and six hundred. Already some two hundred and twenty have been described, but of these the habits of the two hundred are yet to be made known.

Anthidium is a genus of fixed morphology, that is the species rather markedly resemble one another. All have the abdomen destitute of hair above, and brightly ornate with yellowish markings. Notwithstanding this similarity in appearance the habits of the species are quite different. Two distinct methods of nest-building are presented, from one or the other of which the species, at least so far as known, scarcely ever depart. The French entomologist, Fabre, has tersely called these two categories, those of the "Cottonniers" and of the "Résiniers."

The cotton-workers do not show much deviation from the habits of other bees and might be considered to possess the primitive nesting-habit of this genus. Among bees the female either digs a hole in the ground or, more usually, occupies one already dug. Apparently all that is sought is a convenient

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 12.

nidus; the form is of small moment. *Anthidium* has been known to choose abandoned burrows of other bees, or the excavations made by Scarabæid larvæ. There are several cases recorded where even key-holes have been utilized. In other species the nests are placed, not in the ground, but in hollow plant stems. In all these cases the nest is lined with a hard compacted cottony down scraped from the stems of pubescent plants by means of the dentate mandibles of the female.

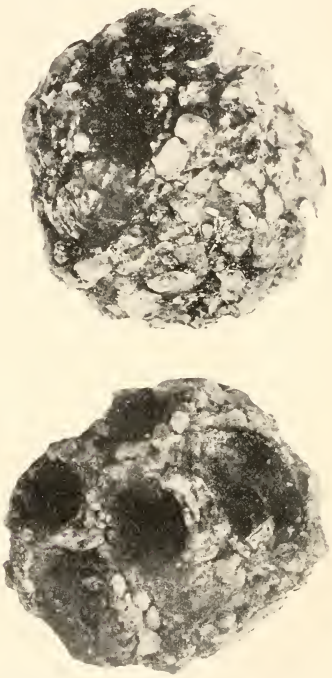


FIG. 1. Nest of *Anthidium texanum* Cress.

With the "Résiniers" the habits are to some extent different. They have the peculiar habit of constructing their nests with the aid of the resin of various conifers. In addition to this some

of the species invariably seek out the empty shells of different Helices wherein to start their future family.

Thus far, the habits of but three of the forty-one North American species of *Anthidium* have been made known. Of these *emarginatum* and *paroselæ* are "Cottonniers," and construct their nests normally in dry sand-banks. The third species, *consimile*, builds among the branches of shrubs or in crevices in rocks and has habits somewhat resembling those of the resin-worker, *texanum*, here to be described.[1]

That *Anthidium* presents the two types of nest construction in both the Old and the New World shows these to be very ancient habits whose divergence occurred at an indefinitely remote period.

While collecting insects in January, on the south side of the Colorado River, a few miles west of Austin (Texas), at an altitude of about eight hundred feet, a nest of what proved to be *Anthidium texanum* was found.

This structure was fastened to a branch of cedar (*Juniperus virginianus* Linn.) about eight feet from the ground. In appearance it was like a small rounded conglomerate with a greatest diameter of twenty-three, and a least of eighteen millimeters. This curious nest consisted of small pebbles of limestone, from one to three millimeters in thickness, securely cemented together with an amber-colored resin, presumably derived from the cedars. Its weight after the bees had transformed was five grammes.

In this mass were six pupal cells. They consisted of a tough, chestnut-brown membrane, more or less transparent so that the pupæ within could be seen. They were four and one half millimeters in diameter, about ten millimeters deep, with a rounded bottom, flat top, and slightly narrowed in width above. The flat top consisted of tougher material, in large part spun in concentric rings around a whitish, conspicuous mammilla. This projection, which is characteristic of *Anthidium*, contained a central hole, and canal to the interior.

[1] In the description of *consimile*, Dr. Davidson did not state of what material the cement was composed. Since then he has informed me that the species builds at times miles from any pines, so that then, at least, other plants probably furnish the resin. Another species, which unfortunately was not identified, was found building in pine resins on San Jacinto Mt.

The arrangement of the pupæ deserves notice. Two were entirely enclosed in the resinous mass except at their mammillated end. The three adjacent were more exposed, all on the same side from the two enclosed cells, and arranged in a row parallel with that of the two, while the sixth, which contained the only male, was quite uncovered and placed on another side of the nest. This also was the last to transform. The five contiguous cells contained females. All the pupæ were oriented alike; also, the head of the pupa was nearest the mammillated end.

FIG. 2. A single pupa cell showing the mammilla.

The first bees, two in number, emerged on May 16th, and immediately crawled back into their cases head first. The next transformed on the 19th, the fourth and fifth on the 25th, while the male did not appear until June 4th. Each imago repeated the habit of the former ones, by crawling head-first into its vacated pupa-case. The adult bees seemed to have eaten the flat end of their envelope in getting out, for no traces of it could

FIG. 3. *Anthidium texanum* Cress.

be found afterwards in the breeding-cage. No cottony pubescence whatever was employed in the nest-construction. The

Californian *consimile* uses granite pebbles as the basis for its nest whereas *texanum* employs limestone, there being no granite at Austin.

The adult bees are quite rare, apparently only two males having been taken previously, by Mr. G. W. Belfrage, in Bosque Co., Texas. These are the types in the collection of the American Entomological Society in Philadelphia, and have been compared with the present specimens to insure the accuracy of the specific determination. The females differ from Cresson's description of the male in having the clypeus largely black; the only yellow occurs in the extreme lateral corners. The sixth abdominal tergite is wholly black. The mandibles also are black and their apical margin is evenly truncate, whereas in the male they are apically emarginate on each side of a median tooth.

Below is appended a list of the species of *Anthidium*, the habits of which are known, and also the papers referring to these. The habits of a number of species are reviewed by Dr. H. Friese in his Apidæ Europæ, Vol. IV. Many interesting notes on the flowers visited by *Anthidium* are given in Knuth's Blütenbiologie, especially in Vol. II., pt. 2, p. 601.

"COTONNIERS."

cingulatum: Fabre, Souveniers Entomologiques, Vol. IV.

diadema: Fabre, Souveniers Entomologiques, Vol. IV.; Rudow, Soc. Entom., 1887–1888.

emarginatum: Davidson, Ent. News, 1895.

florentinum: Rudow, Soc. Entom., 1887–1888; Fabre, Souv. Entomol., Vol. IV.; Rudow, published by the author, Perleberg.

lituratum: Rudow, Soc. Entom., 1887–1888.

manicatum: Rudow, Soc. Entom., 1887–1888; Rudow, published by the author, Perleberg; Verhoeff, Zool. Jahrb., Syst. VI.; Fabre, Souv. Entom., IV.

montanum: Dalla Torre, Entomolog. Nachricht., 1880.

oblongatum: Rudow, Soc. Entom., III.; Xambeu, Bull. Soc. Ent. France, 1896.

paroselæ: Minnie Newberry, Psyche, 1900.

scapulare: Fabre, Souv. Entom., IV.

"Résiniers."

bellicosum : Fabre, Souv. Entom., IV.

consimile : Davidson, Ent. News, 1896.

contractum : Lichtenstein-Montpellier.

latreilli : Fabre, Souv. Entom., IV.

quadrilobum : Fabre, Souv. Entom., IV.

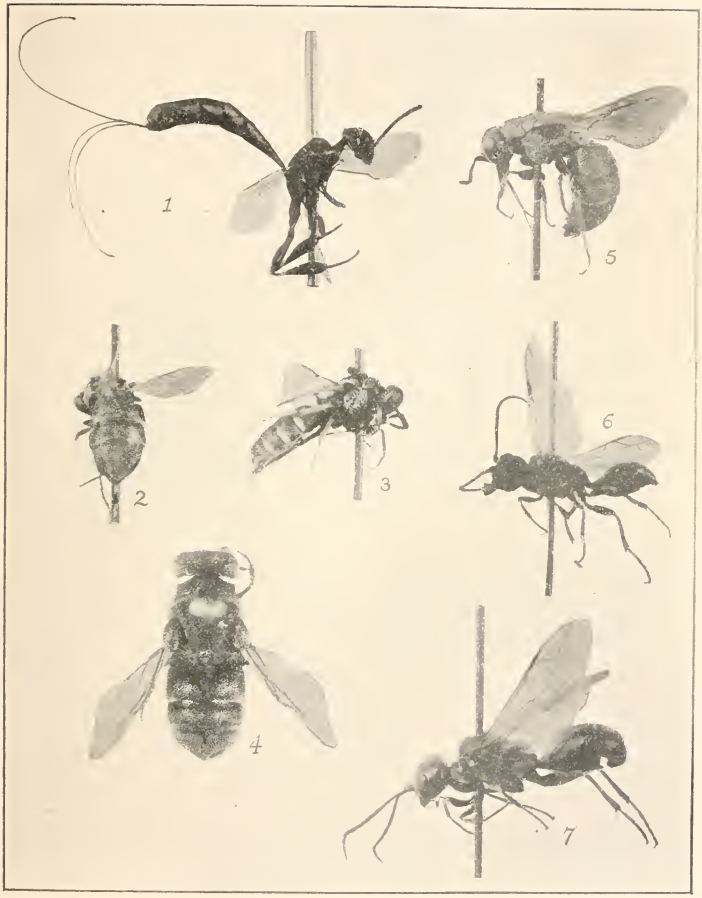
septem-dentatum : Fabre, Souv. Entom., IV. ; Xambeu, Bull. Soc. Ent. Fr., 1896.

stictieum : Lucas, Explorat. d'Algérie, 1846 ; Tosi, Bull. Soc. Ent. Ital., XXIX.

strigatum : Friese, Apidæ Europæ, Vol. IV. ; Kirschbaum Nassanisch. Jahr., 1871.

texanum : Described above.

The University of Texas,
Austin, Tex., Nov. 14, 1900.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Explanation of Plate.

Fig. 1. *Gasteruption pattersonæ*, ♀.
Fig. 2. *Parnopes westcottii*, ♀.
Fig. 3. *Parnopes westcottii*, ♂.
Fig. 4. *Parnopes aglaspidula*, ♀.
Fig. 5. *Parnopes aglaspidula*, ♀.
Fig. 6. *Trigonalys hollensis*, ♀.
Fig. 7. *Trigonalys hollensis*, ♂.

All figures are enlarged; reproduced from photographs made by the authors.

NEW SPECIES OF GASTERUPTION, TRIGONALYS, PARNOPES AND PSAMMOPHILA.[1]

AXEL LEONARD MELANDER AND CHARLES THOMAS BRUES.

GASTERUPTION.

Gasteruption (Fœnus) pattersonæ, sp. nov.

Female.—Length, 11 mm., ovipositor 10 mm. Body ferruginous. Head black. Dorsum of thorax; dorsal line on first abdominal segment, extending nearly to tip of segment; dorsal spots on second and third segments, somewhat indistinct on third; then a dorsal line which widens out, so that it covers the last segment almost completely; piceous. Ovipositor shields very dark fuscous. Legs ferruginous, hind tibiæ slightly darker outwardly and toward apex, and banded with white near base. Clypeus and mandibles ferruginous, the latter black at extreme tip. Palpi rufous, last joint black. Antennæ about as long as head and thorax, fuscous; scape rufous; flagellum black at base and lighter below except on apical joint. Wings hyaline, nervures and stigma fuscous. Head, thorax, and posterior coxæ silvery pubescent. Clypeus quadridentate, fringed with golden hairs. Front punctate above antennæ, and very finely and somewhat obsoletely, transversely aciculate. Prothorax rather deeply and finely punctured. Pleuræ rugose; dorsum and metathorax transversely rugose aciculate. Abdomen impunctured, smooth, not very shining; strongly compressed at base, widest at third segment which is one-half as wide as high. Ovipositor longer than abdomen, alutaceous.

Described from a female specimen, collected by Miss Rose Patterson, August 11, 1898, at San José, California.

This species may be readily recognized by the entirely ferruginous pleuræ, legs, and antennal scape and by the very slight amount of black upon the abdomen. It is the only species recorded from California.

Mr. Ashmead[2] has pointed out the synonymy of *Fœnus* Fabr., and *Gasteruption*, which was described two years previously, viz., in 1796, by Latreille.

TRIGONALYS.

The species of *Trigonalys* from the United States and Canada so far known may be recognized by the following artificial key:

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 11.

[2] Classification of the Ichneumon Flies; *Proceedings United States National Museum*, No. 1206, July, 1900.

Insect almost wholly black..... 2
With conspicuous yellow markings..... 3
2. Abdominal spots larger; body less coarsely punctured; subtegular tubercle black; ♂, 9.5 mm ..... *pullatus* Schuck.
Abdominal spots smaller; body strongly punctured; subtegular tubercle yellow; ♂, 11 mm..... *hollensis*, var. nov.
3. Second abdominal segment fulvous with a large, subquadrate, black spot on each side, and a transverse lateral white spot at tip..... *pulchellus* Cress.
Second abdominal segment black with at least a tranverse yellow stripe..... 4
4. Scutellum wholly yellow; 7 mm..... *sulcatus* Davis
Scutellum somewhat black in middle; larger..... 5
5. Coxæ and femora black; wings with subapical cloud..... 6
Coxæ yellowish, femora dark fuscous; wings fuscous along costa . *costalis* Cress.
6. Head confluently punctured..... *nevadensis* Cress.
Face above antennæ, and vertex impunctate..... *canadensis* Harntgn.

TRIGONALYS PULLATUS Shuck. var. HOLLENSIS, var. nov.

Male.—Length, 11 mm. Black, coarsely punctured, with almost sericeous, short white pile proceeding from each puncture, and covering whole insect. Antennæ with twenty-four joints, black, filiform, slender, the middle joints stouter than the others, the joints evenly decreasing in length to the pointed tip; scape black. Head strongly, closely punctured; antennæ inserted in upper portion of transverse broad depression. Mandibles prominent, broad, with three or four well-marked teeth. Maxillary palpi slender, six-jointed; labial palpi consisting of three stouter joints, the last securiform. Tegulæ piceous; tubercle below tegulæ yellow. The punctation of the thorax is more rugose than that of head or abdomen. Parapsidal depressions well marked, as is also a central longitudinal groove. Scutellum prominent, with median longitudinal groove, and deeply foveate at sides. Postscutellum strongly fossate at sides. Metathorax rugosely reticulate on posterior declivity. First segment of abdomen short, triangular, second largest, four times length of first, third one half length of second, rest of abdomen incurved, not visible from above, fourth and fifth segments subequal, sixth provided with a rough series of small tubercles. At the apex of the abdomen are two short claspers. Second, third and fourth segments each marked with an inconspicuous yellow lateral spot near posterior margin. First and second ventrals equivalent to first and second dorsals, remaining segments much smaller on account of the infolding of the abdomen: second ventral provided with a subapical, flat, median tooth, beyond which the abdomen is deeply notched; last ventrals carinate in middle. Legs marked with yellow as follows: exterior faces of all tibiæ, broadly interrupted on middle pair and narrowly on posterior ones, exterior face of front metatarsi, and exterior two thirds of middle and hind metatarsi. Pectus swollen, deeply, longitudinally grooved. Wings hyaline at base, with a slightly infuscated cloud on apical third: subcostal and externo-

medial nervures strong, piceous, remaining veins weaker, interrupted in places; second and third submarginals subequal, second narrowed towards marginal cell, third subquadrate.

Female.—Differs from male as follows: Length, 8½ mm. Antennæ twenty-three-jointed. Second abdominal segment only with a small yellow posterio lateral spot. Abdomen without the secondary sexual characters of the male, shaped much as in *Nomada*, with four genital filaments, the outer pair of which are fimbriate. Four posterior tibiæ with only a basal yellow band, the hind ones with only slight indications of the striping. The second submarginal is longer than the third and subquadrate.

The two specimens were taken at Woods Holl, Mass., during the latter part of July. They had the peculiar habit, noticed in *canadensis* by Dr. Geo. W. Taylor, of alighting on the leaves of low trees.

The stripes of the four anterior tibiæ show a twisting from the knee towards the front. In the male there is also a small basal stripe almost contiguous to the anterior side of the stripe on the hind tibiæ.

Inasmuch as Mr. Shuckard's three-line description of the color markings of *pullatus* can be applied with quite as much certainty to *Oxybelus quadrinotatus* or even to *Eristalis tenax*, it seems advisable at least to redescribe the present form under the name given. Mr. Wm. H. Ashmead writes that he has a specimen of this rare wasp from Minnesota, which in consideration of the type-locality, North Carolina, shows a wide distribution for this species. The differences given in the table between *pullatus* and *hollensis* are not positively stated. The following are the North American species of *Trigonalys* from Mexico northwards.

pullatus Schuckard,	1841,	N. Car., N. J.,	9.5 mm.	♂
costalis Cresson,	1867,	Mass.,	9. "	♂
pulchellus Cresson,	1867,	W. Virginia,	8.8 "	♂
nevadensis Cresson,	1879.	Nevada,	8.8 "	♂, ♀
canadensis Harrington,	1896,	Victoria B. C.	10.5 "	♀
canadensis Harrington,	1898,	Gabriola Is., B.C.	11.4 "	♂
sulcatus Davis,	1897,	New Jersey,	7. "	♂
hollensis n. var.,	1900,	Mass.,	11. "	♂
			8.5 "	♀

PARNOPES.

In 1874 Mr. F. Smith published the description of *Parnopes chrysoprasina* from North Carolina. Since that time no one has found this insect, though the fact that the species of this genus

are widely distributed is not to be doubted. But four North American species have hitherto been known, and these, with the two new species, may be readily identified by use of the following dichotomy:

Postscutellum excised 2
Postscutellum entire ..... *edwardsii* Cress.
2. Flagellum black; legs metallic; tegulæ green..... *aglaspidula*, sp. nov.
Flagellum and legs reddish; tegulæ in part testaceous 3
3. Face and metapleuræ bare..... 4
Face and metapleuræ with silvery pubescence; wings hyaline..... 5.
4. More or less bronzed; tibiæ brown..... *festitus* Ckll.
Green; tibiæ with greenish tint ..... *chrysopasina* Smith.
5. Postscutellum black, wings hyaline; abdomen green or bronzed; 6.5 mm. *westcottii* sp. nov.
Postscutellum fulvous; apex of wings somewhat infuscated; abdomen bluish; 9 mm..... *fulvicornis* Cam.

PARNOPES AGLASPIDULA, sp. nov.

Female.—Length, 10 mm. Bright metallic blue-green. Rugosely-closely punctured. Nowhere densely pubescent, but with a few scattered white hairs, more closely placed on sides of posterior margins of abdominal segments. Antennæ piceous, the scape with greenish reflections. Face above antennæ smooth, excavated, with impression of an equilateral triangle the base of which lies between the bases of the antennæ and from the apex of which arises a vertical line passing nearly one half way to the anterior ocellus. In *P. edwardsii* there is no such sculpturing. Occipital punctures confluent. Mandibles rather robust; black, except for piceous central space. Clypeus concolorous with face, its black apical margin truncate and devoid of hair. Proboscis reaching to first abdominal segment. Thorax with larger punctures than head, the punctures becoming stronger posteriorly. The median prothoracic impression is almost obliterated, as are also the parapsidal grooves. Between the parapsidal grooves the thorax is almost black, likewise the scutellum and postscutellum. Postscutellum with a narrow incision extending about one third the distance into the disc, its sides straight in one specimen and bowed outwardly in the other. Tegulæ narrow, wholly blue-green, with less strongly-marked punctures than thorax. Metathoracic angles prominent, with reticulate markings. Abdominal segments violet-blue on anterior and posterior margins, green in middle portion; with no apically depressed bands: apical segment with a median carina terminating anteriorly in a narrow, smooth space and posteriorly separating the two subapical oblique grooves, the margin denticulate, with the stronger teeth, about fourteen in number, in a middle series. Venter piceous, smooth. Legs concolorous with body, though gradually lighter in color towards end: knees, apices of tibiæ, and tarsi lighter. Wings infuscated, the veins piceous.

The two females from which the foregoing description was made were taken in Illinois, one in 1897 at Chicago, and the other on August 16, 1899, at Meredosia, in central Illinois, where the fauna and flora are decidedly southern. This is a very distinct species.

PARNOPES WESTCOTTII, sp. nov.

Male.—Length, 7 mm. Bright metallic green varied with coppery reflections. Rugosely punctured. Covered with silvery pubescence as follows: face as far as anterior ocellus densely; metapleuræ densely; sides of scutellum and of postscutellum densely; tegulæ sparsely; suture between prothorax and metathorax sparsely; posterior portions of abdominal segments sparsely, but more densely at the sides; and distal portions of the legs moderately. A few scattered hairs are present on the occiput, thoracic dorsum, coxæ, and femora. Punctures rather irregularly placed, stronger on thorax than on abdomen, confluent behind the ocelli, where they are radially arranged, the rows diverging anteriorly. Antennæ with the scape piceous and flagellum yellowish. Clypeus emarginate, although not so strongly as in *festitus*, yellowish at apex. Mandibles rufous, metallic green at base and black at tip. Proboscis reaching back to the middle of the first abdominal segment. Thorax green, with bronze tint, especially when viewed from in front. Prothorax with a rather well-marked median impression. Tegulæ broad, yellow, disc darker, sparsely punctured. Between the parapsidal grooves the mesonotum is less brilliant. Scutellum and postscutellum piceous, more strongly punctured than the rest of the thorax. Postscutellum excised to nearly its middle, the sinus rounded at the bottom and with a width of about 70 degrees. Sides of postscutellum slightly rounded outwardly. Abdominal segments blue at anterior margin, varying through green and cupreous to piceous on posterior depressed third. This depressed portion is provided with much smaller and confluen, punctures, and with the white pubescence parted on the median line. Fourth segment with two wide subapical oblique grooves separated posteriorly, beyond which it is fulvous: its apical margin denticulate, with seventeen teeth in upper rows, below which is a series of lighter colored and more pronounced teeth, the apical two of which are almost spiniform. Venter shining, uniformly fulvous. Legs fulvous, excepting the metallic green coxæ and femora. The trochanters, knees, and upper surface of anterior femora are lighter. Wings hyaline, the nervures testaceous.

Female.—Length, 6.5 mm. Differs from male as follows: Color, a stronger green, with less of the cupreous reflections. Face broader. Scape of antennæ with greenish tinge. Clypeus not so evidently emarginate, black. Mandibles black except rufous spot in middle. Proboscis reaching as far as last abdominal segment. Occipital punctures not radially arranged. Sides of postscutellum, straight. Apical depressed bands of abdominal segments much narrower. The third segment (apical) has

narrower, shorter, and more oblique, subapical grooves nearer the tip of the segment, its apex with less of the fulvous color. The upper apical teeth less stronger pronounced than in male, lower ones more pronounced. Venter piceous.

This interesting little species, the smallest North American representative of the genus is described from two specimens, the male, taken in California, during August, by Dr. O. S. Westcott and the female during last June at Galveston, Texas, by ourselves. It is not probable that the two sexes represent different species, for the arrangement of the teeth of the apical abdominal segment is not constant in *Parnopes*. The difference in coloration seems largely due to the age of the specimens. The male specimen seems not to be so fully chitinized as the other.

The nearest congener of this species is *fulvicornis*, from Presidio, Mexico. It is readily distinguished by the tabulated differences. A specimen from Washington, in the collection of the American Entomological Society seems referable to this species.

PSAMMOPHILA Dahlbom.

The genus *Psammophila*, established by Dahlbom, in 1842, is now represented by at least six species from America, north of Mexico; and possibly by another species (*violaceipennis*) which was described by St. Fargeau as American. As the species was from Pennsylvania and has not since been recognized, it seems quite possible that the type was not from America. From his description it is no doubt a *Psammophila* and has been included in the table.

Ammophila collaris Cresson, which has been referred to *Psammophila* does not belong there; it is a true *Ammophila*.

The following dichotomy will serve to separate the species:

Body wholly black 3
Abdomen more or less ferruginous 2
2. Pubescence of thorax wholly black 4
Pubescence of thorax more or less gray or white 5
3. Face and clypeus without silvery pile, body polished ..... 3 *luctuosa* Sm.
Face and clypeus silvery pilose, body more or less silvery pollinose.
4 *argentifrons* Cress.
4. Abdomen entirely ferruginous, mandibles wholly black ..... 1 *valida* Cress.
Abdomen in part black 6
5. Petiole short, not extending beyond posterior trochanters, stout species, 22 mm. ..... 2 *grossa* Cress., ♂.

Petiole much longer, smaller and more slender species 8
6. "Wings black, violaceous, dilated part of petiole, second abdominal segment, and anterior border of third, ferruginous" ..... 7 *violaceipennis* St. Farg.
Wings much lighter, petiole black 7
7. Petiole short, not extending beyond posterior trochanters, stout species.
2 *grossa* Cress., ♀.
Petiole extending far beyond trochanters, slender species.
5 *communis* Cress., ♂, ♀.
8. Third submarginal cell small, barrel-shaped, eyes strongly convergent below.
6 *pacifica*, sp. nov.
Third submarginal not barrel-shaped, eyes only slightly convergent, pubescence often dark.....6 *communis* Cress., ♂.

PSAMMOPHILA GROSSA Cresson.

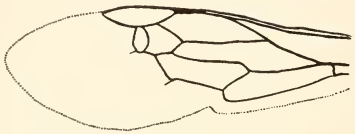
Male.—Length, 22–24 mm. Wholly black, except ferruginous band on mandibles; second abdominal segment below; and narrow bands on third and fourth segments anteriorly above, dilating on the sides and coalescent below; ferruginous; so that second to sixth ventral segments are ferruginous. The dorsal segments also show faint ferruginous bands posteriorly on second to sixth segments, diminishing posteriorly; while in one specimen the black is considerably reduced, until the second and third segments are almost wholly ferruginous. Clypeus and face to considerably above antennæ silvery pubesent. Head, thorax and coxæ bearing long cinereous hairs. Legs white pollinose in certain lights and abdomen slightly so. Clypeus subtruncate, narrowly margined and not dentate; flat below, slightly convex above. Antennæ slightly attenuated toward tip, the apical eight joints each with a sharp carina inwardly. Head very sparsely and rather coarsely punctured. Dorsulum more densely and coarsely punctured. Mesothorax with a short sharp groove anteriorly. Mesopleuræ more densely and coarsely punctate. Metathorax obliquely aciculate on the sides, rugose reticulate dorsally, Legs and abdomen very stout, legs with black spines. Wings light brown, with a violaceous tinge, darker on exterior border. Third submarginal cell oblique, narrowed above. Second submarginal cell receiving the recurrent nervures just before the middle and apex.

Described from two specimens collected at Austin, Texas, during May, 1900.

Although the males differ by their white pubescence from the female described by Cresson, there seems to be no doubt as to their identity, as most specimens of *communis* present the same white pubescence in the males and not in the females. Other specimens of *communis* ♂ collected in Illinois have the pubescence wholly or partially black.

PSAMMOPHILA PACIFICA, sp. nov.

Male.—Length, 12 mm. Black shining, first, second and third abdominal segments bright ferruginous. Clothed with very long pubescence which is white upon the thorax and mixed with black upon the head, especially upon the vertex and face. Head sparsely punctate except around ocelli where it is smooth. Antennæ black, slender, moderately long. Face and clypeus to above antennæ silvery pubescent. Clypeus broadly subtruncate. Mandibles indistinctly annulate with ferruginous. Eyes strongly convergent below, reaching nearly to base of mandibles. Prothorax and mesonotum sparsely coarsely punctured. Pleuræ and sides of metathorax coarsely and densely punctured. Scutellum strongly longitudinally aciculate, the grooves widening out posteriorly to form a row of



FORE-WING OF PSAMMOPHILA PACIFICA.

pits along the posterior margin. Posterior face of metathorax finely transversely aciculate. Coxæ black, clothed with long white hairs which also extend to bases of femora. Tarsi slightly tinged with fuscous, thinly white pollinose. Abdominal petiole black, white pilose anteriorly. Abdomen very slender, first, second and third segments bright ferruginous, remainder shining black. External genital organs fuscous. Their lamellæ with several stiff bristles interiorly and a brush of hairs externally. Anterior wings hyaline, brownish at tip, posterior one hyaline. Marginal cell extending far beyond third submarginal cell, which is less than half as large as the second and barrel-shaped.

Described from a male specimen collected by Miss Rose Patterson at Pacific Grove, California, July 9, 1897.

This species may be readily recognized by the venation of the wings, the strongly convergent eyes, white pubescence and very slender form.

1. *P. valida* Cress.	21 mm.	Colo.
2. *P. grossa* Cress.	22–24 mm.	Texas.
3. *P. luctuosa* Sm.	17 mm.	U. S.
4. *P. argentifrons* Cress.	13–15 mm.	Colo., N. Mex.
5. *P. communis* Cress.	12.5–18 mm.	Colo., Ill., N. Mex., Mass.
6. *P. pacifica*, sp. nov.	12 mm.	Cal.
7. *P* (?) *violaceipennis* St. Farg.	21 mm.	Philadelphia, Pa.

THE ACCESSORY CHROMOSOME—SEX DETERMINANT?

C. E. McCLUNG.

Part I. Observations and Comparisons.

The peculiar chromatic element discussed under this name in several recent papers is one that gives promise of throwing considerable light upon the nature of the chromosomes. So long as all chromosomes of the nucleus were observed to pass through a cycle of changes apparently identical in each case, there was little chance to gain an insight into their interrelations. With the discovery of the accessory chromosome and the recognition of its true chromosomic character, however, there has been offered an opportunity to draw comparisons and so to formulate conclusions which, in time, are certain to materially increase our knowledge of these most important nuclear structures.

In recognition of this fact, and with the hope of hastening such a desirable end, I have devoted some time to the study of the accessory chromosome and have also encouraged students in my laboratory to direct their attention to it. Much material has been collected, and is still being accumulated, in order that as broad a view of the subject as possible could be obtained.

Since, however, the difficulties involved in securing and preparing material from widely different forms would unduly delay the attainment of any comparative results, I have confined my studies largely to the Orthoptera. This has been done in the belief that more substantial good can be derived from a thorough knowledge of a limited group than from a superficial acquaintance with a wider field. Once the basic principles underlying the cellular phenomena of one group are discovered, their recognition in other forms will be rendered much easier.

As a result of the studies so far pursued, it has been found that individual forms rarely present all the details of a problem equally well. Different species excel in the clearness with which certain points are brought out. A feature obscure in one species will appear distinct enough in another while for the elu-

cidation of other structural peculiarities the relations might be reversed. By studying, therefore, an extensive collection of nearly related forms, it is possible to draw a composite outline of a process which will be found applicable in its main features to all the members of the group concerned.

This fact is taken advantage of in the series of studies upon insect spermatogenesis now being pursued by myself and students. Instead of taking one species and endeavoring immediately to make out the entire series of processes which characterize its spermatogenesis, we have considered restricted questions, and have chosen such species as would offer the best facilities for answering these. Thus, in the case of the accessory chromosome, it has been found advantageous to trace its course through the spermatogonial divisions in *Brachystola*, and through the spermatocyte changes in *Hippiscus*. Probably the spermatid transformations will demand another form for their best exemplification.

The danger involved in such a method is the liability which it offers toward accumulating a series of observations upon exceptional or strongly modified types. This has been guarded against, as far as possible, by verifying the appearances of one form by those manifested in others. While the work thus far done indicates considerable variations in the details of the different processes, it does not seem to suggest any wide deviations from general principles, so that the danger of error in the direction of exceptional instances would not appear to be great.

A recognition of the accessory chromosome as such is of very recent date and the literature upon it is therefore not very extensive. And while its chromosomic character has, in a number of instances, been appreciated and even its participation in the supreme division act of the chromosomes in the metaphase noted, the mere resemblance in general features to the nucleoli has sufficed to caused its inclusion in the group of these questionable bodies. A majority of the references to it will accordingly be found in the literature devoted to a consideration of the nucleoli, and in only a few cases will it be found discussed as a chromosome.

On account of the scantiness of the literature, it has been

thought advisable to give here all the probable references to the accessory chromosome in the language of the different authors. It may be possible thus to unify the conflicting opinions regarding the essential features and to leave the field clear for a discussion of the more involved points. Also, in furtherance of the plan previously mentioned of confining the question to a limited area, only the references to insects will be discussed in detail. By this restriction, however, very little material will be excluded since most of the work has been done upon insect testes.

In the following paragraphs will be found all the references—in the literature at my command—to structures which I regard as possibly identical with the accessory chromosome. These will be given chronologically and finally compared and commented upon.

1. The work of Platner ('86) upon the Lepidoptera unfortunately sheds no light upon the nature of the accessory chromosome in that order of insects. Only casual mention is made of the nucleolus and the references do not enable us to gain much insight into its character. The following quotations embrace the principal references: "An letztern Punkten haben auch die Nucleolen ihre Lage. Diese erscheinen selten in der Einzahl, meist findet man deren zwei. Sie sind von ziemlicher Grösse, färben sich stärker und zeigen eine kugelige Form; doch geben meist die von allen Seiten an sie herantretenden Kernfäden ihnen ein unregelmässiges zackiges Ansehen."

2. "Diese (Elemente) färben sich gleichmässig stark mit Safranin und zeigen nicht nur in ihren Dimensionen sondern auch hinsichtlich ihrer Zahl grosse Schwankungen. Unter ihnen befinden sich auch die Nucleoli."

3. Henking ('90) finds in the spermatogonia of *Pyrrhocoris*, in preparations fixed with picro-acetic acid, a nucleolus which is not apparent in those preserved with Flemming's fluid. It appears rounded and of some size, and retains a yellow color while the chromatin stains dark red with carmine. It is observed occasionally to have divided.

4. Concerning the "nucleolus" of the first spermatocyte, Henking has this to say: "Vor allem auffällig ist es an diesem Zellen dass ein grosser Nucleolus zur Ausbildung gekommen

ist. Derselbe tritt bei den verschiedensten Konservirungsmethoden stets sharf hervor. . . . Erscheint der Nucleolus der jüngsten Hodenzellen bei der genannten Methode farblos, so nimmt er nun begierig Farbe auf, ein Verhalten, welches ganz regelmässig ist und vielfach von den beiderseitigen Nucleolus auf dem gleichen Schnitte beobachtet werden kann."

5. The position of the nucleolus is referred to as follows: "In einer Bucht an der Oberfläche der Chromatinkügelchen liegt der Nucleolus eingesenkt."

6. Regarding the constancy of appearance exhibited by this element, he says: "Allein der Nucleolus hat bei den Veränderungen von Kern und Zelle sein Aussehen nicht gewechselt. Er liegt noch wie zu Anfang als rundlicher Körper dem Rande des Kernes angenähert, . . . Der Nucleolus behalt seine Kugelgestalt unverändert bei, während die Chromosomen gewissermassen Pseudopodien aussenden und sich so zu einem Netz vereinigen. Der Nucleolus bietet seinerseits den Pseudopodien keine Ansatzfläschen und bleibt daher isolirt."

7. Zur Zeit der zusammenballung der Chromatinmassen ist er durch seine beträchtlicher Grösse immer noch leicht zu sehn und seine Kugelform macht ihn kenntlich wenn die Chromosomen durch Zusammenfliessen des Chromatins an Volumen ihn zu überragen beginnen. Wenn dann aber die Auflockerung des centralen Haufens anhebt, tritt eine Verkürtzung der Chromosomen ein, wodurch dieselben ihm immer ähnlicher werden. Schliesslich ist er nicht mehr mit Sicherheit heranzufinden. Dafür das er gänzlich rückgebildet wird habe ich gar keine Andeutung erhalten. Er ist so lange in voller Ausbildung deutlich zu erkennen, als ihn seine characteristiche Gestalt vor einer Verwechselung mit anderen Gebildung schützt. Allerdings müssen wir annehmen das er späterhin eine Einschnürung erfahrt da auf einem definitiven Stadium alle Chromosomen eine gleiche Form besitzen.

8. Having thus traced his "Nucleolus" up to its final disappearance among the group of chromosomes in the first spermatocyte, Henking leaves it and goes on to a consideration of the division of these elements. He notes here the peculiar character and behavior of one "pair" of chromosomes which is noticeable

on account of its deep staining power and inertia during division. This latter is so strong as to cause it to remain undivided in the second spermatocyte mitosis, and so to pass into only one of the two resulting spermatids. It is, therefore, as the "Doppelelement *x*." instead of the "Nucleolus *n*." that we trace its further history.

9. After an extended discussion of the subject, embracing an account of the delayed division of the unusual chromosome of the first spermatocyte mitosis and its later behavior, Henking sums up his conclusions in the following words: "Demnach glaube ich sagen zu dürfen: Bei der letzen Theilung der Spermatocyten wird das Chromatin ungleich getheilt, derart, das die eine Spermatide nur 11 Chromosomen erhält, die andere dagegen ausser den 11 Schwesterchromosomen noch ein ungetheilt bleibendes Chromatinelement."

10. With respect to the further behavior of this latter element, Henking observes that "Nur das isolirt Körperchen hält sich davon entfernt, tritt nicht in so einige Berührung mit den 11 Chromosomen."

11. Under the treatment of the spermatid transformations, Henking discusses the participation of his "isolirt Einzelelement" in the following language: "Von der allgemeinen Vertheilung hält sich nur das isolirt Einzelelement zuruck, welches bei der letzten Halbirung der Spermatocyten ungetheilt in die eine Tochterzellen übergegangen war. Es sind somit die, wenn wir so wollen, bevorzugten Tochterzellen auch jetzt immer noch zu erkennen.

12. His final opinion of the element is expressed in the following quotations: "Ich glaube, ein jeder unbefangene Beobachter wird mit mir diesen runden von dem übrigen Chromatin scharf unterschiedenen Körper für das Kernkörperchen ansehen. Damit ergiebt sich aber die wichtige Thatsache, *dass wir zweierlei Spermatozoen erhalten: die einen besitzen einen Nucleolus, die anderen nicht.*"

13. In the summary of his results, he expresses the same thoughts in slightly different language. He says: "Es sind zwei verschiedenwerthige Arten von normalen Samenfäden vorhanden. Die einen enthalten nur 11 chromatische elemente, die

andern ausser 11 chromatischen Elementen auch noch ein einzelnes zuletzt ungetheilt gebliebenes Chromatinelement, welches wahrscheinlich als Nucleolus anzusehn ist."

14. The investigations of Toyama ('94) upon *Bombyx* and several other Lepidoptera, like those of Platner, do not afford us any very definite idea of the accessory chromosome in this order. Several references to nucleoli are made, however, and these I will quote:

15. "The nucleolus (first spermatocyte), lying either in the chromatin mass or outside of it, persists, as is unusual in skein stages of other animals, till to the end of the skein stage shortly to be described. . . . The nucleolus, however, shows no change from the first resting stage till the present stage, and always consists of small chromatic granules imbedded in a less stainable matrix.

16. "In a still later stage the chromatin granules again commence to separate from one another, and the nucleus again presents the appearance shown in Figs. 31 and 32. In most cases two nucleoli are found in the nucleus of this stage. These gradually migrate toward the periphery of the nucleus facing the center of the cyste (rarely, facing the wall of the cyste) and are finally pushed out into the cytoplasm one after the other through the nuclear wall at this point. Placed in the cytoplasm the nucleoli seem to change their quality, since they now stain differently from what they did when they were in the nucleus. This is shown by the use of Hermann's triple staining, by which the nucleolus in the cytoplasm takes a brownish color, while it colors deep red so long as it is within the nucleus. The further fate of the nucleoli in the cytoplasm is not known.

17. "In this stage I have not found any nucleolus in the 'Kernplatte,' while Henking observed it in a spermatocyte of *Pyrrhocoris apterus*."

18. Wilcox ('95) in his studies upon *Caloptenus femur-rubrum* and *Cicada tibicen* finds peculiar nucleolar structures which will be found described in the following excerpts:

19. "Cytoplasm and achromatic nuclear parts were stained green, the chromosomes, nucleolus, and centrosomes red (safranin and victoria-green). . . . In *some* stages the chromosomes were

stained green, indicating that a chemical change takes place in the chromatic substance. But even in such cases the nucleolus was bright red. . . . By this method (Henneguy's) the chromosomes and nucleoli are stained bright red, the individual chromosomes being sharply outlined. . . . During the stages shown in Figs. 49, 51, 52 (spermatocytes of *Cicada*), there appears to be a chemical change in the constitution of the chromosomes. By the safranin and victoria-green method the chromosomes stain red, though not so deeply as the nucleoli. At later stages the chromosomes assume a green color, while the nucleoli continue to stain red. In still later stages the chromosomes again take the red.

20. "One or often two nucleoli are to be seen (spermatogonia of *Cicada*). . . . The cells of *b* (a spermatocyte cyst) each contain one or two bodies which I consider nucleoli, since they react to the stains quite differently from the chromosomes. . . . The nucleolus then moves to the periphery of the nucleus, and appears meantime to have divided into two portions, one of which passes into the cytoplasm, while the other remains in the nucleus; later, both parts appear outside the nucleus and on diametrically opposite sides of it."

21. With this incomplete account, the nucleolus is left by Wilcox. But in a subsequent paper ('96) he takes up the later history of the element and carries it into the spermatozoon. Two paragraphs will give his conclusions.

22. "The body which appears in the vacuole of the nucleus is rather problematical, both as to its origin and its fate. It appears usually as a rod of deeply staining substance, whose longest axis is in the long axis of the vacuole; but the rod may have the form of a crescent.

23. "The tentative conclusion to which I have come with regard to this body is, that it represents the nucleolar substance of the nucleus of the spermatid, and that it subsequently passes into the mass of chromatin, with which it becomes homogeneously mingled. My evidence for this is as follows: Very soon after the second division of the spermatocytes a body is seen in the nucleus. It (*cres.*) lies at first among the chromatic granules of the nucleus, but is distinguishable from any of the latter by its

greater size and deeper color. Then it comes to lie in the vacuole of the nucleus. At length, what I consider its remains are found for some time faintly discernible in the chromatic mass of the head of the immature spermatozoon. In later stages this body is not to be distinguished from the rest of the chromatic mass. I was at first inclined to believe that this body allied itself with the centrosome to help in forming the neck-body, but was soon convinced that this is not true, because I observed that the two parts of the centrosome and this problematical body exist at the same time in the same spermatid. In Figs. 90, 92, and 106 the body in question is seen in contact with the chromatic mass, and in Figs. 103, 104, it is nearly included in the chromatic crescent. Later, as already indicated, it becomes indistinguishable from the rest of the head of the spermatozoon. Accordingly, I am unable to determine whether or not it forms any definitely limited portion of the head."

24. While Henking was the first to discover the unusual behavior of a "nucleolus" in the spermatocyte which, in all of its manifestations but one, corresponds to an ordinary chromosome, Montgomery ('98) deserves the credit for observing that this body is merely a chromosome of the spermatogonia that pursues a somewhat different course from the others. Unfortunately, however, his interest in nucleolar structure led him to denominate it, with Henking, a nucleolus and it is accordingly under the name "chromatin nucleolus" that we shall trace its history through the spermatocytes of *Euchistus*.

25. The persistence of a color reaction in this element that is characteristic of chromosomes in the metaphase first attracts Montgomery's attention. He states in regard to this that "in each nucleus, from the commencement of the anaphase on, one of the chromosomes still retains the red stain characteristic of all of them in the immediately preceding period, and this particular element is destined to become the chromatin-nucleolus, the metamorphosis of which will be described later."

26. Subsequently he discusses the chromatin nucleolus at some length and from this part of his paper I will quote passages bearing upon its name, staining reaction, form, behavior, final disposition, and function. He says:

27. "To return to the chromatin nucleolus. I give this name in order to express its genetic origin, and to distinguish it from the true nucleolus; it differs also from the "karyosomes" found in many cells, which are nothing more than temporarily thickened portions of the chromatin reticulum. . . . But when the chromosomes have become more or less elongated, all of them stain violet (with increasing intensity of color), except one, which remains red (saffranine), and by strong light may be easily distinguished from the other chromosomes. This one is the chromatin nucleolus, characteristic for the spermatocytes. At least one whole chromosome becomes thus metamorphosed; and it is very probable, judging from my observations, that only one becomes thus changed. This chromatin nucleolus retains in all stages up to the formation of the spermatids its red coloration after the use of Hermann's double stain, and so can be easily distinguished from the true nucleolus as well as from the chromatin of the rest stage and anaphase.

28. "The chromatin nucleolus appears to undergo the same changes of form as do the other chromosomes, up to about the synapsis. Then it ceases to elongate, and in the post synapsis gradually commences to assume a spherical form, which is characteristic for it during the telophase and the rest. When it may first be distinguished in the early anaphase, and also during the synapsis, it lies within the nuclear cavity, not in contact with the nuclear membrane; but at the end of the synapsis it gradually takes up a more peripheral position, so that usually during the synapsis, and always in the telophase and rest, it is closely apposed to the nuclear membrane. . . . But in most cells in the synapsis it occurs in the nuclear cavity apart from the chromosomes. In such cases it is found to be usually rod-shaped, often more or less curved, occasionally even lobular; but so great is its irregularity in form, that in no two cases does it have exactly the same shape. . . . Afterwards it either gradually shortens up into the ultimate spherical form, or first becomes constricted at one or more points on its surface, showing then a more or less beaded appearance, and then, by division at these points, breaks into a number of unequal fragments, each of the latter subsequently rounding off. . . . But it is most probable that at first

only a single one is present, *i. e.*, that only one chromosome becomes changed into a chromatin nucleolus: for in the synapsis, when it may best be distinguished from the chromosomes, I have never seen more than one long chromatin nucleolus. From the synapsis on, the surface of the chromatin nucleolus gradually becomes smooth, so that its process of rounding off may be regarded as a mode of concentration of its substance. It finally becomes homogeneous, quite different from the microsomal chromosomes.

29. "These phenomena show us the remarkable process of the casting off of an entire chromosome, which is itself possibly a mode of chromatin reduction; and in the two spermatocytic divisions we shall find that the chromatin nucleolus does not again become a chromosome. . . . There are only two other thinkable modes of origin of the chromatin nucleolus: 1) that it be extranuclear in origin, or 2) that it be a secretion of the chromatin.

30. "In the spermatocytes of *Harpalus* a chromatin nucleolus has been seen by me, besides the true nucleolus; and judging from the observations of authors on various objects it would seem that such a structure is generally characteristic of spermatocytes. Thus Moore ('95) found in Elasmobranchs about the beginning of the synapsis, 'a curious secondary nucleolus surrounded by a vacuole, which, so far as I can ascertain, is in these fishes characteristic of this change.'

31. "Thus the true nucleolus passes from the periphery toward the center of the nucleus, the chromatin nucleolus in the reverse direction.

32. "The chromatin nucleolus lies now in contact with the nuclear membrane, and is rounded with the exception of that side flattened against the nuclear membrane.

33. "In the early prophases the chromatin nucleolus becomes rounded, but at first retains its central clearer globule. At the loose spireme stage it commences to grow smaller, at the same time losing the central globule. . . . The decrease in size continues until the end of the loose spirem, when a dimension is attained which is approximately uniform for the chromatin nucleoli of all cells; one or more of the smaller bodies, which arose as

fragments of the original chromatin nucleolus, may still be seen in the nucleus, and often up to the monaster stage. At the time when the chromosomes have attained their definitive form, it usually becomes likewise elongated and dumb-bell-shaped; in the majority of cases it appears to assume this form before the nuclear membrane disappears. Thus it looks like a diminutive chromosome among larger ones. As the true chromosomes now stain with saffranine it likewise resembles them in coloration. This peculiar structure acted like a nucleolus in the rest stage, but in the monaster is destined to lie in the equator among the chromosomes, where it also becomes divided in metakinesis, and so terminates in acting like a chromosome, as at the commencement it has been formed from one.

34. "In a few cases, so few that they must be considered abnormal, a whole undivided chromosome passes into a second spermatocyte, but I have met with only two or three such cases. Henking found in *Pyrrhocoris* and later in some other cases, that the second spermatocytes receive an unequal number of chromosomes, *i. e.*, that one of them may frequently if not usually receive a whole undivided chromosome; either *Pyrrhocoris* shows a marked peculiarity in this respect, or else Henking had mistaken either a yolk globule or a chromatin nucleolus for an undivided chromosome. . . . Each second spermatocyte appears as a rule, if not always, to receive a half of the original chromatin nucleolus.

35. "The 7 chromosomes and the chromatin nucleolus gradually become arranged in the equator of the spindle, their axis parallel to the latter, and the plane of their constrictions perpendicular to it. Then follows the metakinesis, with a consequent transverse (reduction) division of all the chromosomes, and apparently in most cases of the chromatin nucleolus, with the result that each daughter cell (spermatid) receives 7 daughter chromosomes and 1 daughter nucleolus."

36. Paulmier ('99) records in his observations that "These interesting bodies (the small chromosomes) were first recognized in the equatorial plate of the spermatogone divisions in the form of two chromatin masses very much smaller than the chromosomes and connected with them by chromatin bands. In the

resting spermatogones they appear as two rather indefinite bodies, staining with the chromatin stains, and apparently not breaking down to the same extent as the rest of the chromatin. During the period of spermatocyte growth they come to view again in the synapsis stage, as a single body. This body has at first an irregular shape, then it elongates, splits longitudinally, and again transversely, thus forming a tetrad in essentially the normal manner, though passing over many of the stages which the other tetrads go through. During these stages it shows a decided difference in its staining reactions, taking at all times a deep black stain with hæmatoxylin, while the rest of the chromatin is scattered irregularly and stains gray. It always lies close against the nuclear membrane. In the first spermatocyte division it lies in the center of the ring of chromosomes, and divides somewhat before the others. The two parts are connected with each other by two threads precisely as are the normal tetrads.

37. "In the second spermatocyte division it goes bodily over into one of the two daughter-nuclei without showing any traces of division, beyond a slight elongation due to the pull of opposing spindle fibers. In a slightly later stage it again shows its difference from the other chromosomes by retreating as far as possible from them. Soon the disintegrating force overtakes it, and it becomes indistinguishable from the others.

38. "I think that we may say without hesitation that this body is not a true nucleolus, a possibility precluded by its different staining reaction, the constancy of its occurrence, and by its division. We find also in addition to it a perfectly normal, true nucleolus in both the resting spermatogones and spermatocytes.

39. "I agree with Henking that it is chromatin, and that the nuclear substance is thus divided unequally. This body is absent in one-half of the spermatozoa which nevertheless, as far as we know, produce normal descendants. I would make the suggestion that it is degenerating chromatin; in other words, that these small chromosomes, or idants (to adopt for the moment Weismann's terminology), contain "ids" which represent somatic characters which belonged to the species in former times, but which characters are disappearing. The "ids" which represented these characters are much slower in disappearing than the characters

themselves, and persist as the two small chromosomes of the spermatogones. These then undergo a pseudo-reduction and form a tetrad which is unable to complete the second spermatocyte division."

40. My own early views concerning the accessory chromosome, as manifested in the Locustidæ, may be found expressed in the following quotations from my first paper ('99) upon the subject:

41. "As it first appears in the spermatogonia of *Xiphidium fasciatum*, there would be no hesitation in calling it a nucleolus except for its unusual situation on the surface of the nuclear vesicle. It is a small, irregularly rounded body, and lies immediately under the nuclear membrane. Before the division figure is established, however, it takes on the form of a thread which becomes 'U'-shaped. Still further contraction ensues, and by the time of the metaphase the thread has become very short and thick and is bent in the middle with an obtuse angle so as to resemble a boomerang. At this time, it may be observed lying at one side of the circle of chromosomes arranged in the equatorial plate, and plainly distinguishable from them by reason of its greater length. From the pole the chromatin appears as a broad, fenestrated plate, and the accessory chromosome is indistinguishable from the ordinary ones. Because of the rapidity of the division none of the anaphases are to be seen, but in the telophases the ordinary chromosomes of the cell may be seen grouped in the typical manner at the two ends of the spindle, while extending down towards the equatorial plate from each mass is a half of the boomerang-shaped body which has been divided longitudinally in the same manner as the ordinary chromosomes.

42. "In the resting stage of the spermatocyte that succeeds the appearance just described, the accessory chromosome again appears as it did in the resting stage of the spermatogonia, and would easily be taken for an ordinary nucleolus. Soon, however, it commences to assume a thread-like form which finally results in the production of a long 'U'-shaped body, a form that is retained during the greater part of the spireme stage. In this condition it lies at the surface of the vesicle and stains in its usual intense manner. Concurrently with the formation of the 'rings'

from the spireme thread, it commences to shorten and grows into the form of a horseshoe, and is finally to be distinguished from the chromatic rings only by its deeper staining quality and by the smoothness of its outline. In the formation of the mitotic figure of the first spermatocyte division, it assumes its position on the outside of the group of chromosomes as it did in the spermatogonial division, and again has the boomerang shape that marked its appearance in the early figures. When the chromatin separates and moves to the two poles, the accessory chromosome divides longitudinally and presents the appearance of two horseshoes with their rounded ends in contact. In the second spermatocyte division apparently the same process is followed.

43. "The recently formed spermatids possess a nucleus in which the ordinary chromatin is extremely scant and very weak in staining power, while the accessory chromosome shows as prominently as ever and stains in the same uniform manner. It is not easy to trace out the part that the different elements of the nucleus take in the formation of the spermatozoon, but in the light of present knowledge it appears as if the accessory chromosome was prominently concerned in the formation of the head.

44. "In seeking to point out the features that characterize this peculiar nuclear element, perhaps the most striking thing to be noticed, is the almost uniform staining power exhibited.

45. "Thus there seems to be no reason to suppose that the accessory chromosome of *Xiphidium* arises by the direct transformation of one of the ordinary ones, although such a change may be possible. This does not argue against the chromatic origin of the body, however, for it is almost certainly modified chromatin, but in *Xiphidium* it arises during the resting stage and may represent derivative substance from one or all the chromosomes."

46. A later, more detailed, study of various members of the Acrididæ ('00) gave me a much better insight into the nature of the accessory chromosome and the result of my observations upon its behavior during the spermatocyte divisions are summarized in the following words:

47 "As a result of the last spermatogonial division, the much reduced daughter cells are each provided with the somatic num-

ber of chromosomes. All but one of these rapidly disintegrate and from their substance produce the spireme of the first spermatocyte. One persists in its original form and, assuming a peripheral position, continues to stain as does a chromosome of the metaphase. During metakinesis it is divided like other chromosomes. This is the accessory chromosome.

48. "The accessory chromosome is a constant and important element of the germ cell. It arises, in the Acrididæ, from a spermatogonial chromosome, and from that time forward maintains a separate and distinct existence. During the prophases, when occur the profound changes that result in the production of a nucleus with only half the ordinary number of chromosomes, this structure stands aloof and self-contained. With the establishment of the mitotic figure of the first spermatocyte, however, it takes its place with the other chromatic elements and becomes indistinguishable from them henceforth until the spermatids are formed. Here it again becomes distinct and conspicuous."

49. Up to the present time, we have learned nothing of the real origin of the accessory chromosome. That it is one of the spermatogonial chromosomes that passes over into the spermatocytes without taking part in the formation of the spireme is pretty well established; but as to the manner of its appearance in the spermatogonia, we have no knowledge. Sutton ('00) is the only one who has traced the history of the element at all fully in the spermatogonia. Concerning its first appearance and its subsequent history in this generation of sex cells he says, "In *Brachystola*, the accessory chromosome appears probably in the first, and certainly in the third, secondary spermatogonial division, and goes through precisely the same changes in each cycle up to the last.

50. "It may occasionally be distinguished from the other chromosomes in the metaphase and anaphases by its granularity and greater length, though it always divides like the others, and in the actual process of division, as a rule, is indistinguishable from them. In the telophase it constructs its own membrane just as the others do, but soon becomes sharply contrasted with them by the deposition of its chromatin in a diffused condition upon the inner surface of its vesicle (vesicular chromosome), and

also by the fact that from this point to the following metaphase the cavity of its vesicle remains distinct from that formed by all the others. In these stages the vesicle of the accessory chromosome may lie on any portion of the nuclear membrane proper; in some cases occupying a position between the 'fingers' or sacculations of it (Fig. 34). This vesicular stage is of comparatively long duration, and is followed by a receding of the chromomeres from the membrane to form a chromatic rod, first loose, rough, and granular, but gradually growing more slender, and compact, and often becoming twisted (Fig. 16). It also betrays a longitudinal split at a stage later than that at which a similar occurrence is observable in the ordinary chromosomes.

51. "From the middle prophases to the telophases, its conduct is so similar to that of the other chromosomes that it would hardly be an error to speak of the cells of these stages as having two nuclei, one having a single chromosome and the other a large number, the small nucleus always lagging slightly behind the large one. This lagging of the accessory chromosome is nicely shown in Fig. 21, already alluded to, where in a stage just before the metaphase the nuclear membrane is seen to be dissolved while that of the body in question is still intact.

52. "In the last or transformation division of the secondary spermatogonia, some differences are noticeable in the behavior of the element under consideration. The vesicular stage seems to be of slightly longer duration, and while, after its close, the same condensation of the chromatin takes place, no longitudinal split appears until the late prophases or 'ring stage' of the spermatocyte; and in the course of the earlier prophases of the growth period its vesicle gradually becomes fused with the nuclear membrane, its outer half completing the smooth contour of the latter, while its inner portion projects into the nuclear cavity. In this stage it has the appearance of an irregular vesicle filled with a homogeneous, darkly staining liquid or semi-liquid body, suspended within the membrane of the nucleus. Later it again becomes granular, and in the first spermatocyte division divides as it did in the spermatogonia.

53. "The resting stage of this element, as shown by its staining violet with Flemming's three-color stain, is what I have called

the vesicular stage, and this only, since at all other times it stains a bright red. The absence of the formation of a spireme at any stage in the development of this element is paralleled by Henking's description of the normal process in all the chromosomes in the spermatogonia of *Pyrrhocoris*.

54. "Perhaps the most important thing to be gained at present from the knowledge of the behavior of the accessory chromosome in *Brachystola* is the light which it throws upon the question of the individuality of the chromosomes. In the first place, the fact that it is a true chromosome, though different from the others, is shown by its staining reactions and by the parallelism between its development in the spermatogonia and that of its more generally recognized fellows. Although it shows a tendency to lag behind the other chromatic bodies, the only radical difference between the two is the absence of the loose spireme in the accessory, and this is paralleled, as shown above, by the normal process of all the chromosomes of *Pyrrhocoris*, according to the statement of Henking. The apparent radical difference in the case of the vesicular stage is, in reality, only a matter of degree, for it frequently happens that the ordinary chromosomes, in going into the diffused condition, leave a very appreciable hollow in their centers (Fig. 30). Apparently there is, for some reason, a necessity that the chromatic granules of the accessory come into closer relation with the cytoplasm than those of its mates, and the result is their deposition upon the vesicle itself—this vesiculation being really a substitute for the loose spireme so conspicuously lacking.

55. "Now, if it be admitted that the body *is* a chromosome, inspection quickly shows us that it maintains throughout the spermatogonial divisions, as well as in those that follow, an indubitable independence, being enclosed, in all stages except those of actual division, in its own individual membrane. Having, then, one of the chromosomes which preserves its individuality in this way, and seeing the other chromosomes enclosed for a part of their development in similar individual vesicles, which only become intercommunicating by absorption of a part of their walls, have we not a right to suppose that at one time they too enjoyed the same independence as their more exclusive mate? In other

words, have we not a right to suppose that their phylogeny is paralleled by their ontogeny? If this be granted, then we have at least more ground for belief in the individuality of the chromosomes than if we had never known of a time when they were of necessity independent."

56. Of interest in connection with the question of the general distribution of the accessory chromosome are the observations of Miss Wallace ('00) upon the male germ cells of the spiders. The general facts of the case are found in the following quotations from her preliminary paper: "In this spider the peculiar chromosome is conspicuous in the late spermatogonic stages, and in the prophase of the first spermatocyte, its peripheral position in the nucleus making it easy to observe. Its origin in the spermatogonia has not yet been traced, but a gradual change of form has been made out in the early stages and, suffice it to say here, that in all of them it appears to be double.

57. "In the monaster of the first spermatocyte the accessory chromosomes are easily distinguished from the others by their sharpness of outline, slightly greater affinity for staining reagents and above all by their eccentricity of position. They are always found on the periphery of the spindle and often near the periphery of the cell. It is a curious fact that in the majority of cases both of these elements are found nearer one pole than the other."

58. Regarding the unequal distribution of the accessory chromosome to the spermatozoa, she is unable to speak definitely. Her observations on this point are stated in the following words: "In the spider the position of the two chromosomes nearer one pole gives the impression that this unequal distribution occurs in the first spermatocyte division. One thing, however, opposes this interpretation and that is that in the monaster of the second spermatocyte division two elements are again found in eccentric position but of half the size of those found in the preceding cell-division. The position nearer one pole might mean merely delayed distribution but there is not yet at hand sufficient data from which to draw a conclusion."

59. In the latest edition of his work upon the cell, Wilson ('00) takes cognizance of the investigations being pursued by insect

spermatologists and practically adopts Paulmier's views concerning the accessory chromosome. We find in his summary that "A comparison of the foregoing results indicates that the small tetrad (dyad) corresponds to the extra chromosome observed by Henking in *Pyrrhocoris*, and perhaps also to the 'accessory chromosome' of *Xiphidium*. Whether it corresponds to the 'chromatin nucleolus' of *Pentatoma* is not yet clear. The most remarkable of these strange phenomena is the formation of the small tetrad, which seems to be a non-essential element, since it does not contribute to all the spermatozoa. Paulmier is inclined to ascribe to it a vestigial significance, regarding it as a degenerating chromosomes which has lost its functional value, though still undergoing in some measure its original morphological transformation; in this connection it should be pointed out that the spermatocyte nucleolus, from which it seems to be derived, is represented in the spermatogonia by *two* such nucleoli, just as the single small tetrad is represented by two small chromosomes in the spermatogonia mitoses. The real meaning of the phenomena is, however, wholly conjectural."

Because of the greater prominence of the element in the spermatocytes, observations upon its occurrence and changes have been more numerous and accurate than in the case of the spermatogonia. During the prophase of the first spermatocyte, particularly, the appearance of the element is so striking as to render its oversight impossible. Regarding the main features distinguishing it, there is a convincing agreement in all the published reports and these speak further for the morphological exclusiveness so plainly manifested by the element in the spermatogonia. There are some slight discrepancies in the accounts of its very early appearance but concerning the later stages there appears to be no confusion.

From the Hemiptera (¶ 27) and the Orthoptera (¶ 47), we learn that the chromosomes of the last secondary spermatogonia, with the exception of one, break down rapidly into their constituent chromomeres and that these enter at once into the formation of a spireme—at first thin and fine but later coarse and granular. The exception to this process is the accessory chromosome. It is, in the beginning, somewhat irregular in outline

but quickly condenses its substance and becomes homogeneous, transparent, and sharply outlined. It early assumes a position upon the periphery of the forming nuclear vesicle and maintains this during all the prophase. Throughout this period, and the remaining stages of its existence, it persistently stains according to the reaction exhibited by chromosomes of the metaphase. It is thus, in both its chemical and physical properties, strikingly different from the remainder of the chromatin. It may be said, therefore, that it is a chromosome of the previous generation (*i. e.*, one formed from the spireme of that generation) which exists as such while its fellows pass through the prophase of another mitosis.

So far as the first spermatocytes are concerned, this is the only point at which the accessory chromosome differs from the others. When metakinesis occurs, all the chromatin elements divide at the same time and in the same way. The participation of the accessory chromosome in this act is generally conceded (¶ 33, 36, 47), but the exact process has not yet been observed because no form has been studied where the element stands out conspicuously enough to be noted. From the nature of the element, however, the only reasonable thing to expect would be that it should divide as it has done in all previous mitoses, *i. e.*, longitudinally.

With regard to the action of the accessory chromosome in the second spermatocyte mitosis, there are two opinions. One is that it takes part with the ordinary chromosome in the act of division (¶ 34), the other that it passes undivided into only one of the two resulting cells (¶ 9, 12, 13, 37). Examination of the literature shows that the weight of evidence, so far as observations are concerned, supports the latter view. In addition to this, the fact that it differs from the other chromosomes must be taken into account. When it divides in the first spermatocyte it has finished the entire act of separation begun for it in the prophase where it was formed. It has no need, therefore, to divide in the second mitosis where the other chromosomes complete the separation of the chromatids formed in the prophase of the first spermatocyte. Thus, when the origin of the element is taken into consideration, a phenomenon at first apparently inexplicable

according to the usual laws governing cellular activities is seen to be in strict accord with them. The variation from normal conditions is, accordingly, manifested by those chromosomes which emerge from the spireme of the first spermatocyte instead of by the one which fails to enter into it.

At the completion of the second spermatocyte mitosis the apportionment of the chromatin to the germinal elements is accomplished and the further changes in the spermatid would seem to indicate that there is no longer the necessity for the chromosomes to maintain their separate identities. This, because the chromatin first becomes diffuse and later condensed so as to form the head of the spermatozoon where there is no distinction of parts. In the spermatid where the accessory chromosome is present, there is no apparent difference in the behavior of the nuclear elements. The accessory chromosome runs the course of the ordinary chromosome and sooner or later becomes indistinguishable in the homogeneous mass of the spermatozoon head.

It is, therefore, impossible to trace the location of the accessory chromosome to any portion of this nuclear mass. The fact is apparent enough, however, that it does remain a part of the nuclear contribution to the mature element (¶ 12, 13, 23, 37, 43) and does not go to form a part of the archoplasmic derivations as Wilcox was at first inclined to believe (¶ 23). The merging of the chromatin elements into one mass makes it impossible to go further in the investigation of the accessory chromosome at this point. We can hope to know more about it only by learning its part in fertilization of the egg. As a result of the action of the accessory chromosome in one maturation mitosis, this fact, at any rate must be apparent, viz: that there are two kinds of spermatozoa; those *with* the accessory chromosome and those *without*. Beyond this is speculation only, but with accumulated observations on many forms it may not be long until we are able to reach a definite conclusion regarding the exact function of this well defined element.

From the different observations, I hope (1) to bring out the essential features which characterize the accessory chromosome, (2) to show the extreme probability of its universal occurrence among insects, (3) to outline its history in the different cell gen-

erations of the testis, and (4) to suggest a theory in explanation of its function.

Confirmation of the statement that most references to the accessory chromosome would be found under discussions of nucleolar structures has just been given in the quotations from various papers. When a reason is sought for the classification of such a purely chromosomic element with this heterogeneous group of bodies it is difficult to find any that is sufficient. Interest in other problems has, perhaps, induced investigators to concentrate their attention elsewhere and as a result the accessory chromosome has been assigned .relationships entirely foreign to its true nature.

I have, in previous papers ('99, '00), given my reasons for regarding it as a chromosome, so that I shall not have to go much into detail on this point. It would seem sufficient to show that the element *is* a chromosome of the spermatogonia (¶ 27, 41, 47, 49–53) and that it divides in a subsequent spermatocyte mitosis as a chromosome (¶ 33, 36, 42, 47, 48) in order to insure its classification as such. Yet in full recognition of these conditions Montgomery (¶ 27) calls it a nucleolus and insists (¶ 29) that it never again (*i. e.*, in the spermatocytes) becomes a chromosome. While Henking fails to detect the origin of his "nucleolus" from a chromosome of the spermatogonia he recognizes its chromatic character and its participation in the act of metakinesis and even occasionally calls it a "chromatinelement" (¶ 13).

Wilcox (¶ 19) is very careful to note the staining reaction of the nucleolus and that of the chromosomes. This he finds to be identical except in certain stages, and in these, it is the chromosomes proper that weaken in their affinity for the basic anilines while the "nucleolus" consistently reacts to them with the true chromatin reaction. But in considering the "nucleoli" of the spermatocytes (¶ 20) he regards them as such because "they react to the stains quite differently from the chromosomes." Just in what respect this difference lies is not quite manifest from the text, but it is apparently in being uniform in staining instead of variable.

In his early paper Wilcox ('95) does not trace the final history

of the element so carefully as he does in a later one ('96). Here, even after more extended investigation, he is uncertain "both as to its origin and its fate" but inclines to the belief that it is "nucleolar substance" despite the fact that it does become "homogeneously mingled" with the chromatin. As will be noted (¶ 23), he was at first inclined to consider it related in some way to the centrosome, but as a result of more careful study decided that this was a mistake.

In addition to noting the obvious staining reaction and peripheral position of the element in the prophase of the spermatocyte, Paulmier also observed its behavior in the spermatogonial divisions (¶ 36) and subsequently in the different phases of the spermatocytes (¶ 37). As a result of a recognition of its very apparent chromosomic character he agrees with me in calling it a chromosome, but prefers to speak of it as the "small chromosome." I have already pointed out in a previous paper ('00)[1] my reasons for regarding this as a misnomer on account of its usually being larger than the other chromosomes so need not again refer to the subject. Thus, we have as a result of the latest and apparently most accurate work upon the Hemiptera, the history of a small chromosome that puts it in almost complete agreement with the behavior of the accessory chromosome in the Orthoptera.

From the preceding statements of different investigators, it will be apparent, I think, that there is in the spermatocytes of all insects so far studied an unusual nuclear element which is characterized (1) by a remarkable uniformity in staining power, similar to that exhibited by chromosomes in the metaphase; (2) by a continuous peripheral position during the spireme stage, at least; (3) by an isolation from the chromatin reticulum and nonparticipation in its changes; and (4) by fission during metakinesis after the manner of chromosomes. In addition to these features which have generally been recognized, there are others which material of exceptionally good character has rendered apparent to several observers. Of these I wish to speak later but desire here only

[1] ¶ 3, p. 85; latter part of ¶ 3, p. 86; and ¶ 5, p. 89; were attached to the proof as footnotes but were included by the printer in the body of the article. It thus happens that on page 89 a reference to Paulmier's last paper ('99) appears before a criticism of his earlier one ('98).

to show the great probability of the general distribution of the structure among insects.

The published reports of almost all investigators certainly speak very strongly in favor of such a supposition, but in order to assure myself by personal observation that views of such a character were correct, I examined representatives of the different families of the Orthoptera and became convinced that I was right so far. Later, material from the Hemiptera, Neuroptera, Coleoptera, and Lepidoptera was examined only to confirm the opinion that the element in question is a constant character of the insect testicular cells. The recent work of Miss Wallace upon the spider ('00) would seem to indicate that there is no doubt of its presence in the Arachnids, and with its determination in this class the probability of its general occurrence in the Arthropods is largely increased. I may also say in passing that some hurried examinations of vertebrate spermatocytes lead me to believe that the accessory chromosome is likewise present here.

If it be conceded that we are dealing with a common element of the sperm-forming cells, it must also be admitted that there exist extensive variations in its appearance and manifestations. To do this, however, is no more than to concede what is known to be true of all other chromosomes so that such an admission can in no way impair the standing of the accessory chromosome as a constant and important nuclear element. It will therefore be understood that the outline history of its behavior in the different cell generations, which I intend to give, will apply only in general particulars. Its purpose is more to show the importance of the element than to postulate a type.

In the spermatogonia, our knowledge of the accessory chromosome is due almost entirely to Paulmier and to Sutton. The former author notes in a general way (¶ 36) that in the metaphase of the early cell generations there are present two smaller chromatin bodies which, during the breaking down of the spermatogonial chromosomes to form the spireme, do not suffer any very extensive dissolution. Later these appear as a single body in the prophase of the spermatocyte. Montgomery makes no mention of their behavior earlier than the telophase of the last

spermatogonial division but agrees with Paulmier's view concerning their later changes.

Sutton, on the contrary, has devoted his entire attention to the spermatogonial divisions and has given us a fairly complete account of the changes taking place in *Brachystola* (¶ 49–55).

It is probable that this object presents an extreme view of the accessory chromosome. From an inspection of Sutton's preparations and a comparison with other forms, however, I am strongly inclined to the behalf that it is merely a very marked example of a normal process. We may, therefore, take the course followed by the accessory chromosome in the spermatogonia of *Brachystola* as representative since it is all that we have, at present, to base our knowledge upon.

Even with this we have no hint, as to the real origin of this problematical body—a point of great importance. It is hoped that more extensive collections of material will render it possible to learn something of this, but the problem is one of considerable difficulty owing to the compact nature of the early cells and their apparent irregularity in arrangement. A favorable object may be found, however, in which the structure will stand out clearly enough to be accurately studied, and in this event we may discover the conditions determining the setting apart of this one chromosome from all the others.

Sutton was first able to distinguish the accessory chromosome in cysts of eight or sixteen cells. Here, as in the spermatocytes, it seems to be removed as far as possible from the influence of the ordinary chromatin. This is accomplished by an enclosure in a separate vesicle which, as Sutton observes, may almost be regarded as a separate nucleus (¶ 51). A distinct existence is maintained during all the stages when a possible exchange of material between the accessory chromosome and the other chromosomes might be accomplished. Only after the chromosomes are definitely established as independent bodies are the barriers removed and then only long enough to permit the act of metakinesis to take place.

During the period intervening between the acts of division, the conduct of the accessory chromosome parallels that of the nucleus containing the remainder of the chromatin (¶ 50). A

vesicle is formed, the chromatic substance is deposited upon its wall in intimate relation with the cytoplasm, a concentration ensues and a definite chromosome is produced.

These changes, it seems to me, are easily explainable if we regard the conditions under which the processes operate. The divisions of the spermatogonia are rapid and continuous and every factor concerned is subordinated to filling the follicles with spermatogonia as quickly as possible. This, of course, requires a rapid increase in amount of chromatin including that of the accessory chromosome, and so the anabolic processes are facilitated by bringing the chromatin into a position where it can best derive its nourishment from the cytoplasm. The vesiculation of the accessory chromosome is merely an incident, the isolation of the element being the end sought.

Throughout the divisions of the secondary spermatogonia this process continues until the accessory chromosome of, let us say, the primary spermatogonium has been apportioned to each of the many cells that are now ready to transform into spermatocytes, and during this time it has practically been as independent as if it were the chromatin of a separate nucleus.

Part II. Theoretical Considerations.

In seeking an explanation for the unusual phenomena connected with the history of the accessory chromosome in the male germ cells, it is most natural to surmise the existence of a phylogenetic significance. In the spermatagonia what amounts to practically two nuclei in each cell is strongly suggestive, in mere general features, of the appearances manifested in the Protozoa where both macro- and micronuclei are present. The accessory chromosome might be homologized with the micronucleus which serves as a medium of exchange between the organisms during the act of fertilization, but it would be extremely difficult to trace any parallelism between the macronucleus and the real chromosomic vesicle of the spermatogonia. I do not, therefore, believe that we can look in this direction for an explanation of the peculiar character exhibited by the accessory chromosome.

Nor do I believe that there is the least basis for Paulmier's theory that the structure is a degenerating chromosome. There

are many facts which argue against it. Of these I should like to speak in some detail, since the theory they controvert is the only one yet advanced upon the proper basis that the element is a chromosome and not a mere nucleolus.

Paulmier considers the element a chromosome in the process of disappearing from the species and reaches this conclusion after observing that in the last spermatocyte mitosis it fails to divide and is thus unequally apportioned to the resulting spermatozoa. Before considering a theory based upon so unusual a phenomenon as this, it would be well to make certain that it is an actual and common occurrence. That it is must be granted, I think, after the work of Henking, Paulmier, and myself, upon so many different forms, has shown it to be of such wide distribution. Granting this, then, there remains to be examined the validity of the assumption that the act presages the final extinction of an element.

In combating the suggestion of Paulmier, I shall make use of the evidence offered in the different cell generations, commencing with the spermatogonia. Every instance, according to my interpretation, shows facts strongly incompatible with this author's view. I would suggest in this connection that the extreme importance of the structure is unmistakably manifested by its course in the spermatogonial divisions. What could more strongly emphasize the special importance of an element than to have it set apart in a separate vesicle while its fellows are provided with one common investment? Morphologically, this is simply raising the accessory chromosome to the rank of a nucleus coordinate with the one commonly present in a cell. Its careful and uniform division during the mitoses of all the spermatogonia suggests anything but an unimportant structure. Had we no further refutation of the degeneration theory than that afforded by the spermatogonia, it would, I think, be sufficient. The evidence of the other cell generations, however, strengthens this position and is well deserving of attention.

From the spermatogonia, each spermatocyte receives one chromosome (the accessory) which through all the subsequent stages exists as a chromosome and never suffers extensive disturbance of its chromomeres either for the purpose of metabolic activi-

ties or for the possible exchange of mutual influence with the other chromosomes. Montgomery assigns the origin of the accessory chromosome to a single spermatogonial chromosome, in the Hemiptera, and I have clearly traced it to the same source in the Orthoptera. It must be regarded, therefore, as a *single* element and as the possessor of *two* chromatids, after its longitudinal division, and so differs from the other elements which are constituted of *four* chromatids. This is a matter of considerable importance as will appear when we consider the division of the spermatocytes.

The period which witnesses a breaking down of the spermatogonial chromosomes and the construction of a thin chromatin thread from their chromomeres is marked by changes just the reverse on the part of the accessory chromosome. It enters the prophase as a definite body with a staining reaction that is constant and marked. These characters it maintains until it becomes indistinguishable in the spermatid.

Meanwhile the other chromosomes of the spermatogonia are lost in the substance of the spireme, in which condition their chromomeres exist in relations far removed from those prevailing in the component individual chromosome. This spireme stage is one of extreme importance to the structures it involves. In many cases, as has frequently been pointed out, it is a phase occupying relatively, and sometimes actually, a long period of time. During its continuance, profound changes take place in the nucleus as a result of which the chromatin emerges in the form of chromosomes the like of which we are unable to find in any other cells of the body. Instead of having two chromatids at the time of the metaphase, each of these has four. Instead of being as numerous as those of the spermatogonia, there are but half as many. Throughout all the time involved in the production of these fundamental differences, the accessory chromosome has existed quite apart from the field of mutual influence in which the other chromosomes operate. It is thus apparent that it has its characters fixed, not in the generation which witnesses its division but in the previous one. In other words it is a spermatogonial chromosome which divides in the spermatocyte mitosis.

From this it will be apparent enough why there is an undivided chromatin element in one spermatocyte mitosis. It is but a single chromatid and so cannot be separated into halves. If, therefore, Paulmier bases his theory upon this phenomenon (as it appears he does) it would seem that he has a very insufficient foundation for it. True it is that he considers the element a tetrad and so would be more justified in his conception, but the evidence he brings forward in the proof of this is not convincing. Montgomery clearly recognizes its unit character in other members of the Hemiptera, Sutton traces it through the spermatogonia of the Orthoptera as a single chromosome, and my own observations are positive as to its valence. The weight of evidence is therefore strongly against Paulmier on this point—the essential one in his theory. Moreover, I might point out that the division of the elements has already been accomplished in the prophase of the first spermatocyte and only their separation remains for a succeeding metaphase. The active agents here are the archoplasmic fibers, so that failure to act in unison with the other cell structures would reflect upon their vigor rather than upon that of the chromosomes.

Again, the great regularity of the divisions by means of which exactly one half of the spermatozoa are unprovided with the element would seriously weaken any assumption of degeneration. If the usual course of degenerating structures were followed, it would demand great irregularity and uncertainty in the occurrence of the unequal division, whereby varying numbers of the spermatozoa would be marked by the presence of the undivided element. In none of the forms studied is there any suggestion of an indeterminate and indefinite division, and in the absence of this, Paulmier's theory loses its strongest support.

It is also pertinent to ask whether the dropping of specific characters would take place by the elimination of an *entire chromosome*, if so whether this would occur in the germ cells, and, if in the germ cells, why in the last generation?

In view of all the objections advanced, I believe it would be impossible for Paulmier's hypothesis to maintain its ground without the support of numerous others equally difficult to base upon observed facts. It will be necessary on this account to

look elsewhere for an explanation of the various phenomena involved in the problem. I shall therefore venture to advance a theory which has been suggested to me by a careful study of the structure in various species in the hope that an early elimination of the improbable factors of the question will bring us closer to the true explanation.

In offering a theory to account for the function of the accessory chromosome, I do so with considerable reluctance, for I realize how little real general knowledge we have of this structure. It seems to me, however, that something is necessary to concentrate the interest of spermatologists upon the fundamental character of this most suggestive chromatin element, and I know no better way of aiding in this than by publishing the working hypothesis with which I have attacked the problem.

This has led me into the field of theories concerning sex and its determination, but I have tried to avoid any more extensive discussion than is necessary to outline, in a preliminary way, the opinion I hold concerning the meaning of the accessory chromosome. Even with this reservation I have nevertheless been obliged to go further afield than I should desire with our present knowledge as a guide. I can only hope that my excursions may accomplish a measure of the purpose for which they were undertaken.

Briefly stated, then, my conception of the function exercised by the accessory chromosome is that it is the bearer of those qualities which pertain to the male organism, primary among which is the faculty of producing sex cells that have the form of spermatozoa. I have been led to this belief by the favorable response which the element makes to the theoretical requirements conceivably inherent in any structure which might function as a sex determinant.

These requirements, I should consider, are that: (*a*) The element should be chromosomic in character and subject to the laws governing the action of such structures. (*b*) Since it is to determine whether the germ cells are to grow into the passive, yolk-laden ova or into the minute motile spermatozoa, it should be present in all the forming cells until they are definitely established in the cycle of their development. (*c*) As the sexes exist

normally in about equal proportions, it should be present in half the mature germ cells of the sex that bears it. (*d*) Such disposition of the element in the two forms of germ cells, paternal and maternal, should be made as to admit of the readiest response to the demands of environment regarding the proportion of the sexes. (*e*) It should show variations in structure in accordance with the variations of sex potentiality observable in different species. (*f*) In parthenogenesis its function would be assumed by the elements of a certain polar body. It is conceivable, in this regard, that another form of polar body might function as the non-determinant bearing germ cell.

(*a*) If we accept the theory that the chromatin is the bearer of hereditary qualities, there could be little doubt regarding the necessary chromosomic character of a sex determinant. Sex being an elementary characteristic of protoplasm, it would be firmly established in the hereditary basis along with metabolic activity, irritability, etc., and if any argument were needed at all it would be a general one, not concerned immediately with the question under discussion, but with the broader one suggested. It will therefore be assumed that the chromatin is this basis. This being true, it will only be necessary to point out that the work of a majority of investigators definitely proves that the accessory chromosome *is* a chromosome, and its standing in this respect is established.

(*b*) With regard to what would theoretically be required of a chromosome whose function should be the determination of sex, it is probable that almost every investigator would hold an opinion differing in some respects from those entertained by others. What I can suggest in this connection will therefore be merely tentative and an expression of my own views. One thing, however, would seem to be necessary; *i. e.*, that the determinant should exist in the cells until they are definitely established as elements of either an ovary or of a testis.

If it be that the production of male elements is a sign of katabolic conditions, or, in other words, of those that make a greater demand of energy expenditure upon the developing cell, then it would seem most natural that the determinant should be for the purpose of carrying the transformation beyond the production of

ova to spermatozoa. It would therefore be a necessary content of the cells until they had passed through the stages of development beyond that at which they might pause and become laden with yolk or, in other ways, postpone the period of maturation. It is conceivable that the production of four functional cells from one spermatogonium would call for the employment of more energy than would the formation of one functional egg from an oögonium, especially since many cells contribute their substance or support in the upbuilding of the egg.

Accordingly, it would be most reasonable to expect the presence of the determinant in the latest possible stage consistent with its equal distribution to half the spermatozoa. This we find to be the case with the accessory chromosome which regularly occurs in all the cell generations up to the last and is only withheld, finally, from half of the spermatids. By its consistent course in this respect, the accessory chromosome plainly manifests its intimate influence upon the germ cells of which it is a part, and most strongly suggests a relation to sex determination. It may further be pointed out in reference to this relation, that during the multiplied spermatogonial divisions, the accessory chromosome exhibits a somewhat distant attitude toward the remainder of the chromatin, and it is only at the time of the definitive spermatocyte divisions that it comes to be an intimate member of the cell nucleus. In what manner it is borne from the fertilized egg to the testis of the embryo we do not know, and, lacking this knowledge, are placed at a considerable disadvantage for a proper appreciation of its real character.

(*c*) A most significant fact, and one upon which almost all investigators are united in opinion, is that the element is apportioned to but one half of the spermatozoa. Assuming it to be true that the chromatin is the important part of the cell in the matter of heredity, then it follows that we have two kinds of spermatozoa that differ from each other in a vital matter. We expect, therefore, to find in the offspring two sorts of individuals in approximately equal numbers, under normal conditions, that exhibit marked differences in structure. A careful consideration will suggest that nothing but sexual characters thus divides the members of a species into two well-defined groups, and we are

logically forced to the conclusion that the peculiar chromosome has some bearing upon this arrangement.

I must here also point out a fact that does not seem to have the recognition it deserves; viz, that if there is a cross division of the chromosomes in the maturation mitoses there must be two kinds of spermatozoa regardless of the presence of the accessory chromosome. It is thus possible that even in the absence of any specialized element a preponderant maleness would attach to one half the spermatozoa, due to the "qualitative" division of the tetrads.[1]

(*d*) As I elsewhere suggest, it is most appropriate that the sex determinant should have its locus in the spermatozoa. These elements are most commonly freed from any close relation to the parent organism at maturity, and thus lose the opportunity to receive from it any bias toward the production of an unusual proportion of the one sex or the other as environmental conditions might require. It is otherwise with the ova. They are usually retained by the maternal organism in such intimate relation to it that surrounding conditions might easily imprint their demands upon them. Even up to the time of fertilization the female elements are so placed as to react readily to stimuli from the mother. Here they are approached by the wandering male elements from which they may choose—if we may use such a term for what is probably chemical attraction—either the spermatozoa containing the accessory chromosome or those from which it is absent. In the female element, therefore, as in the female organism, resides the power to select that which is for the best interest of the species.

(*e*) The strength with which sex is established in different species of animals is variable. Moreover it is a fact of common observation that all cell elements vary widely in different animals. We should not be surprised to find, then, that a determinant would exhibit marked varieties of form which might even be carried to the extreme of its entire suppression as a definite element. Incomplete as are the observations upon the behavior

[1] It is suggestive that in all those cases where there appears to be no cross division of the chromosomes in maturation, nothing like the accessory chromosome has been noted. This would seem to be some indication that there might be two types of division.

of the accessory chromosome in various species, enough evidence is forthcoming to show wide departures from anything that might be considered a typical form. And here it is that it may be possible to secure more or less definite information with regard to the meaning of the accessory chromosome. If a large number of observations show variations that parallel well-marked instances of unusual sex characters, then greatly increased probability will attach to the theory I have advanced.

(*f*) Concerning the bearing of parthenogenesis upon the problem of sex determination, we know little. In eggs, no structure comparable to the accessory chromosome has yet been observed and the presence of any such element is extremely improbable. But it is known that different sexes come from parthenogenetic eggs, and in the familiar example of the aphides, these are produced in strict response to environmental demands.

Parthenogenesis, however, is regarded as a degenerate method of sexual reproduction in which polar bodies perform the function of the spermatozoa. Sex might, therefore, be determined by the particular polar body that restored the needed amount of chromatin to the egg, for these, like the spermatozoa, would be of two kinds where a reduction division took place in the process of maturation. These facts would indicate an element of truth in Minot's view regarding the meaning of the polar bodies. In respect to this matter, however, we have only theory to guide us and must wait for more thorough study of the question.

The suggested hypothesis affords a reasonable basis for a number of theories that have been advanced and supported upon empirical data. Among these are Thury's and Düsing's on the time of fertilization; the ones relating to the nutrition of the parents and embryo; and possibly others in which age or "comparative vigor" is assigned as the influential factor.

In general, I would point out, my theory confirms these by showing that the condition of the ovum determines which sort of spermatozoon shall be allowed entrance into the egg substance. In this we see an extension, to its ultimate limit, of the well-known *rôle* of selection on the part of the female organism. The ovum is thus placed in a delicate adjustment with regard to surrounding conditions and reacts in such a way as to best sub-

serve the interest of the species. To it come the two forms of spermatozoa from which selection is made in response to environmental necessities. Adverse conditions demand a preponderance of males, unusually favorable circumstances induce an excess of females, while normal environments apportion an approximately equal representation to each of the sexes.

Those theories regarding sex determination which contain any element of truth within them will be found dependent upon this principle. It is expressed by Geddes and Thompson in these words: "But the general conclusion is tolerably secure—that in the determination of the sex, influences inducing katabolism tend to result in production of males, as those favoring anabolism similarly increase the probability of females." The authors just cited clearly recognize that we must consider the sexual elements in the light of their elemental structure and function when the final explanation of sex is sought. They say: "That the final physiological explanation is, and must be, in terms of protoplasmic metabolism, we must again, however, remind the reader."

The *rôle* that I have suggested for the accessory chromosome in no way changes the ordinary conception of the part played in sex determination by the various observed factors, but it does offer some tangible means by which to correlate these and to fix the nature of their participation.

The conception of two forms of sexual elements which would be operative in the determination of sex is not new. It has been assumed on purely theoretical grounds that there are two kinds of ova, one of which, in the event of fertilization develops into a male organism while the other under similar conditions gives rise to a female. This theory is dismissed by Geddes and Thompson on the ground that the two forms of ova have never been observed and for the further reason that later influences might possibly change the earlier tendency.

The latter objection would prove fatal to any theory which located the determination of sex in a structural difference of the germinal elements. I do not consider this position well taken for reasons that I will give later. The more serious objection lies in the fact that, so far as observation has gone, all eggs of a species are practically alike. It is also to be depreciated because

of the fact that it reverses the ordinary relations of the elements and removes the power of choice from the female.

We have in the case of the spermatozoa, however, the observed fact that there are two essentially different forms and that they are present in equal proportions. No other feature, save sex, separates the resulting offspring into two approximately equal groups. By exclusion then, it would seem that the determination of this difference is reposed in the male element.

There are, I am aware, certain observations upon the determination of sex with which my hypothesis does not seem to agree. Some of these I should like to mention in order to suggest possible explanations or reasons for regarding them within the limit of error set by our present knowledge of the subject. These objections may be suggested by the following questions :

Is sex potentiality—by which I mean the tendency of the species to perpetuate itself in individuals of two sexes of approximately equal numbers—a constant and uniform factor prevailing throughout all classes of animals ? Is sex determined at the time of fertilization ; if so, is such determination absolute, or may it be changed by varying conditions ? Under the unusual circumstance of parthenogenesis, will it be possible to reconcile a theory which postulates the presence of a determinant in the male element with the fact of the entire absence of this element in unisexual reproduction ?

An answer to the first question is not difficult. It is a matter of common observation that all animals are not alike in their methods of reproduction. In the insects, for instance, it is known that certain forms invariably produce young after the sexual method and that parthenogenesis never occurs ; in others, parthenogenesis is the common method and sexual union of male and female only an infrequent occurrence ; while in yet others, one sex is produced by fertilized eggs and the other sex from those unfertilized.

The logical conclusion to be drawn from these facts is that sex, *per se*, is not an unchangeable attribute of organisms but is an adaptation of the species to secure the most favorable conditions for its perpetuation. Given favorable conditions of environment and aphides will reproduce indefinitely with only one sex as a repre-

sentative of the species. Adverse conditions, on the contrary, cause the appearance of the male form which then shares with the female the representation of its kind

Again, some species are influenced by favorable external conditions to such an extent as to cause, not the entire suppression of the male form, but only its subordination in numbers. Finally, there are forms where the numerical proportion of the sexes is preserved with only slight variation even under great extremes in environment. The first case we would consider as an example of a weak, the second as one of moderate, and the last as one of strong, sex potentiality.

Let it be granted, then, that the demand for sexual representation is not equally strong in all species. It follows that we may expect to find corresponding variations in the method by which sex is determined. Such forms as exhibit a ready response to environmental conditions will certainly be more easily influenced even at a late stage of development than would the more stable forms at the beginning.

On this account, an answer to the second question could not be a simple one. It is very probable that, in certain species, sex is determined at the time of fertilization and can not be altered by any later influences. Conversely, it has been experimentally proved that the proportion of sexes may be materially altered by changed nutritive conditions operating upon larval forms, or may possibly be changed several times in the same individuals. But because Yung raised the proportion of females from a normal one of about 56% to the unusual one of 92% in the sexually unstable tadpoles of the frog, it does not follow that in all forms sex is such a variable factor. It is simply an evidence that sex is not a fixed attribute of organisms and that in this particular case it is extremely unsettled. By no means can it be taken as an argument that sex may not be established in the act of fertilization.

In refuting this view, moreover, we are not forced to rely entirely upon negative inferences. In the case of the honey-bee, it has long been known that sex depends solely upon the matter of fertilization. From the impregnated eggs come the females, queens or workers as circumstances dictate; from the unim-

pregnated eggs always males. This fact Dzierzon demonstrated by observation in 1853, and the absence of spermatozoa from the eggs which develop into drones has very recently been proven in the laboratory of Weismann by the use of modern cytological methods.

A further proof, although inferential, is that afforded by "true" twins, in which case it appears that the sex of the two individuals is always the same. If sex were established at the time of fertilization of the ovum, then sex would be shared along with the other qualities possessed by the normal individual that would have developed from the ovum under ordinary conditions. In case sex were not established at the time of impregnation, it would be natural to expect the two sexes to be occasionally represented in one birth because of the inequality of nutrition in the embryos or for other reasons.

Sex, then, *is* determined sometimes by the act of fertilization and can not be subsequently altered. But between this extreme and the other of marked instability there may be found all degrees of response to environment. It must accordingly be granted that there is no hard-and-fast rule about the determination of sex, but that specific conditions have to be taken into account in each case. The objection that Geddes and Thompson raise against the possibility of two forms of eggs, viz., that it is a useless adaptation on account of the fact that subsequent conditions may determine sex in some cases, is not a valid one in general. Such *may* be the case in some instances, but such *is* not the case in others.

Finally, with respect to the evidence to be derived from parthenogenesis, it should be remembered that we are here dealing with a practical suppression of sexuality and it is to be expected that extensive modifications of the ordinary process will follow. If the egg takes upon itself all the functions commonly exercised by it in conjunction with the spermatozoön, it must be that the determination of sex is included. This, in some instances, is a final choice on the part of the ovum and ever afterward one sex only is produced by it; again, however, it maintains a responsive attitude toward environments and gives rise to the sex most needed by the species. It is to be hoped that the

very promising field opened up by the work upon artificial parthenogenesis will throw much light upon these vexed problems.

LABORATORY OF ZOÖLOGY AND HISTOLOGY,
UNIVERSITY OF KANSAS, January 1, 1901.

SUPPLEMENT.

During the period of a year and a half that has elapsed since the completion of the foregoing article, a number of important changes in, and additions to, our knowledge of the accessory chromosome have been made. These are noted in another paper, "The Spermatocyte Divisions of the Locustidæ," soon to be published, so that extended reference to them will not be given here. For the sake of completeness, however, I deem it proper to make brief mention of such as affect the main points of this contribution.

First, I may observe that the exact character of the unequal division of the accessory chromosome in the spermatocytes of the Orthoptera has been established. As may be noted in ¶ 42, I could not determine the behavior of this element in *Xiphidium* with certainty. From its absence in large numbers of spermatids, though, I was inclined to support Henking's view that it remained undivided in one of the spermatocytes. In other genera of Locustidæ, *Orchesticus*, *Anabrus*, *Microcentrum*, and *Scudderia*, I have since been able to demonstrate with certainty that the accessory chromosome divides but once in the spermatocytes. Here, unlike *Pyrrhocoris*, the second spermatocyte mitosis witnesses the separation of the chromatids of the accessory chromosome. The end result is, nevertheless, the same in each case.

Further confirmation of the fact may be found in the work of R. de Sinéty upon other species of Orthoptera, in which he records exactly similar processes. I feel safe in stating it as established therefore, that in the Orthoptera—and in the Hemiptera—the accessory chromosome is normally present in exactly one half the spermatozoa.

With regard to the general distribution of the accessory chromosome, I may state that a student in this laboratory, Mr. M. W. Blackman, has been able to demonstrate its presence in the Myriapoda where it evinces, in connection with all the other cell

elements, a tendency to depart from typical appearances. Montgomery has investigated the spermatogenesis of *Peripatus* and declares that the "chromatin nucleolus" is not present in that form. His evidence, however, I regard as not convincing in this respect.

Credit is assigned Montgomery (¶ 24) for the discovery that the accessory chromosome is merely a spermatogonial chromosome that comes over unchanged into the spermatocytes. This must now be withdrawn, for, at present, he accepts the views of Paulmier that it is formed by synapsis from differentiated elements of the spermatogonia. That it *is*, on the contrary, the same in both cell generations, at least in the Orthoptera, is shown by the work of Sutton (¶ ¶ 49, 52).

Perhaps the most important advance in our knowledge of the accessory chromosome pertains to its relation to the other chromosomes of the cell. During the early investigations upon it, the tendency was to consider it widely removed from the type chromosome, but we are now beginning to perceive that practically its only divergence consists in its isolation. This feature is most pronounced in the prophase of the first spermatocyte where the element bears some little resemblance to a nucleolus—enough, in fact, to have induced several investigators to so call it. But, as has already been shown, this is merely superficial, and later researches upon the Locustid cells have brought to light the fact that the accessory chromosome forms a close spireme of its own, and so parallels the activities of the ordinary chromosomes at the point where it seemed most to diverge. We may therefore regard the accessory chromosome as practically normal in its behavior throughout the different cell generations of the testis up to the point where it is thrown into prominence by the unusual action of the remaining chromosomes during the pseudo-reduction. It may well be, as previously suggested, that the distinction bestowed upon the accessory chromosome at this time is due to its fidelity to the type form of division which, at this point, is abandoned by its fellows.

Regarding the theory of its function advanced in this paper, I can say only that it has, if anything, been strengthened by later researches, and more nearly explains the phenomena involved than any other that has been conceived.

BIBLIOGRAPHY.

Carnoy.
'95 La cytodiérèse chez les Arthropodes. La Cellule, Vol. 1.

Cholodkovsky, N.
'94 Zur Frage über die Anfangsstadien der Spermatogenese bei den Insecten. Zool. Anz., Vol. 17.

Cheshire, F. R.
'86 Bees and Bee-keeping.

Griffin, B. B.
'99 Studies on the Maturation, Fertilization, and Cleavage of Thalassema and Zirphæa. Jr. Morph., Vol. 15.

Geddes and Thompson.
'95 The Evolution of Sex.

Häcker, Valetin.
'97 Ueber weitere Uebereinstimmungen zwischen den Fortpflanzungsvorgängen der Tiere und Pflanzen. Biol. Cent., Vol. 17.

Henking, H.
'90 Untersuchungen über die ersten Entwickelungsvorgänge in den Eiern der Insecten. 2. Ueber Spermatogenese und deren Beziehung zur Eientwickelung bei Pyrrhocoris apterus M. Z. wiss. Zool., Vol. 51.

Henking, H.
'92 Idem 3. Specielles und Allgemeines, ibid., Vol. 54.

Ischikawa.
'91 Studies of Reproductive Elements. Spermatogenesis, Ovogenesis and Fertilization in Diaptomus sp. J. Coll. Sc. Imp. Univ. Japan, Vol. 5.

McClung, C. E.
'99 A Peculiar Nuclear Element in the Male Reproductive Cells of Insects. Zool. Bull., Vol. 2.

McClung, C. E.
'00 The Spermatocyte Divisions of the Acrididæ. Kan. Univ. Quart., Vol. 9, No. 1.

Montgomery, T. H., Jr.
'98 The Spermatogenesis in Pentatoma up to the Formation of the Spermatid. Zool. Jahrb., Vol. 12.

Montgomery, T. H., Jr.
'98 Chromatin Reduction in the Hemiptera : a Correction. Zool. Anz., Vol. 22.

Moore, J. E. S.
'95 On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs. Quart. J. Micr. Sc., Vol. 38.

Paulmier, F. C.
'93 Chromatin Reduction in the Hemiptera. Anat. Anz., Vol. 14.

Paulmier, F. C.
'99 The Spermatogenesis of Anasa tristis. Jr. Morph., Vol. 15.

Platner, G.
'86 Die Karyokinese bei den Lepidopteren als Grundlage für eine Theorie der Zelltheilung. Internat. Monatsschr. Anat. Histol., Vol. 3.

Rath, O. vom.
'92 Ueber die Reduction der chromatischen Elemente in der Samenbildung von Gryllotalpa. Ber. naturf. Ges. Freiburg, Vol. 6.

Rath, O. vom.
'92 Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris Latr. Arch. mikr. Anat., Vol. 40.
Rath, O. vom.
'95 Neue Beiträge zur Frage der Chromatinreduction in der Samen und Eireife. Ibid., Vol. 4.
Sutton, W. S.
'00 The Spermatogonial Divisions of Brachystola magna. Kan. Univ. Quart., Vol. 9, No. 2.
Toyama, K.
'94 On the Spermatogenesis of the Silkworm. Bull. Coll. Agric. Imp. Univ. Japan, Vol. 2.
v. la Valette St. George.
'97 Zur Samen- und Eibildung beim Seidenspinner (Bombyx mori). Arch. mikr. Anat., Vol. 50.
Wagner, J.
'96 Beiträge zur Kenntniss der Spermatogenese bei den Spinnen. Arb. Nat. Ges. St. Petersburg, Vol. 26.
Wallace, Louise B.
'00 The Accessory Chromosome in the Spider. Anat. Anz., Bd. 18, Nos. 13 and 14.
Weismann, August.
'00 Ueber die Parthenogenese der Bienen. Anat. Anz., Bd. 19, Nos. 20 and 21,
Wilcox, E. V.
'95 Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen. Bull. Mus. Comp. Zool. Harvard Univ., Vol. 27.
Wilcox, E. V.
'96 Further Studies on the Spermatogenesis of Caloptenus femur-rubrum. Ibid., Vol. 29.
Wilcox, E. V.
'97 Chromatic Tetrads. Anat. Anz., Vol. 14.
Wilson, E. B.
'00 The Cell in Development and Inheritance. New York. 2d Ed.

THE METALLIC COLORS OF FEATHERS FROM THE NECK OF THE DOMESTIC PIGEON.

R. M. STRONG.

The so-called metallic colors and iridescent effects exhibited by many feathers have been variously explained by different writers. In general they are recognized as diffraction phenomena peculiar to the barbules.

If one observes the neck of a gray domestic pigeon by reflected sunlight, the feathers on the sides will appear bright metallic-green when the angles of incidence and reflection are small. But when the angles are large, the same region has a purplish appearance. Under other conditions a dull brown color prevails. A single feather gives metallic colors only from the distal exposed portion.

The hypotheses based on the supposed presence of striæ or ridges, supported by Haecker ('90) and others are seen at once to be inapplicable to this case when one finds that the feather may be rotated through a whole circle with essentially the same color effects for given angles even from individual barbules. Furthermore, a careful microscopic study of the barbule surface shows that irregularities such as striæ, ridges, pits, knob-like elevations, etc., are not frequent enough when sufficiently small to produce grating effects, and in fact are not normal occurrences.

On comparing barbules giving metallic colors with barbules from the same barb in a region of non-metallic colors, striking modifications are found. The barbules from the region where metallic colors do not appear are of the typical form, with a proximal flattened region, a longer attenuated portion, and well-developed barbicels on the distal barbules. They present their dorsal margins upward in a dorsal view. There is a comparatively sparse pigmentation with the typical rod-shaped granules of melanin found in feathers.

In the region of metallic colors, one finds the proximal barbules essentially like the distal barbules and flattened throughout their extent. The attenuated portions characteristic of typical

barbules are absent as are also the barbicels. There is a very heavy dark pigmentation of the dorsal halves of the barbules and they are arranged so that they present their flattened surfaces upward in a dorsal view. The dorsal margins rest upon the median regions of the next more distal barbules and the ventral margins are thereby hidden from view. The arrangement suggests, somewhat, two rows of shingles overlapping each other on either side of a median axis.

On sectioning these barbules, I find an outer transparent layer less than one micron in thickness, which encloses cell cavities more or less closely packed with *spherical* granules of melanin pigment less than one micron in diameter. The greater part of this pigment is in the dorsal half of the barbule, the part visible in a dorsal view of the feather.

For several months I held strongly to the view that the thin transparent layer produced the well-known interference colors of thin plates, as was supposed by Altum ('54, '54a) and Brücke ('61). The exceedingly uniform size and unusual shape of these pigment granules seemed too significant, however, to warrant an unqualified acceptance of the thin-plate hypothesis. Usually, melanin granules become more or less generally fused together into irregular masses or are imbedded in the horn substance of the feather. In the barbules which give metallic colors, however, the spherical granules retain their individual form. Cross sections frequently show them scattered about outside the barbule section and they seem to have been simply packed in the cell cavities of the barbules with little or no cementing substance to hold them together.

In the feather germ, I find typical pigment cells or chromatophores which produce typical rod-shaped granules. But after these granules have passed into the cells composing the fundaments of the barbules which are to have metallic colors, they are transformed into spherical granules.

By a fortunate manipulation of apparatus, recently, I was able to view barbules by strong reflected sunlight while using a Leitz No. 7 objective. A beautiful pattern of gleaming spheres was presented to my eye. Each pigment granule appeared to be diffracting light and all the colors of the spectrum were visible in the field. On the broad surface of the barbule, where the

angles of incidence were small, the predominant colors were yellow and green, especially green. On the margins where the angles of incidence were great, the prevailing colors were reds and purples.

The colors of the feathers described, when observed without a microscope, are very apparently mixed colors. The greenish effects are produced when light strikes the broad surfaces of the barbules and is reflected with a small angle of reflection. The reds appear only when light falls with a large angle of incidence on the pigment granules of a margin or elevated portion of a barbule.

We seem to have a clear case of Newton's rings where each pigment granule comes in contact with the outer transparent layer.

This is a preliminary statement of my results. Another paper describing them in greater detail with the aid of figures is in process of publication.

April 1, 1902.

BIBLIOGRAPHY.

Altum, B.

'54. Ueber den Bau der Federn als Grund ihrer Färbung. Journ. für Ornith., Bd. 2, pp. xix–xxxv.

Altum, B.

'54a. Ueber die Farben der Vogelfedern im Allgemein, über das Schillern insbesondere. Naumannia, Jahrg. 1854, pp. 293–304.

Brücke, E.

'61. Über den Metallglanz. Sitzungsberichte d. Kais. Akad. in Wien. Math. Naturw. Classe, Bd. 43, Abth. 2, pp. 177–192.

Haecker, V.

'90. Ueber die Farben der Vogelfedern. Arch. f. mik. Anat., Bd. 35, pp. 68–87, Taf. 4.

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STUDIES IN EARTHWORM CHLORAGOGUE.

WM. J. RICE.

The dearth of knowledge concerning the physiological significance of earthworm chloragogue and the opportunity offered for the study of the subject, are the main reasons for the work here presented, which has been carried on under the direction of Professor James G. Needham.

I have found only one article having an important bearing on the subject of this paper, one by Thomas Schaeppi,[1] which was found after this research was practically completed. It may be stated that in several respects a marked similarity exists between the chloragogue of *Ophelia radiata* and that of the earthworm, especially as to color, strong resistance to acid and alkaline reagents and the probability that the chloragogue is excretory in function rather than secretory.

Lumbricus herculeus has served for the most part as material for study. As much as was possible of the work was done with freshly collected material. But when, during the winter months, fresh material was not available, worms were used which had been collected in the late fall and, to insure a thrifty condition, preserved alive for winter use under favorable conditions of temperature and moisture. The worms were found to thrive best in temperature varying from 40° to 60° F. Extremes of heat were quickly fatal. It was found necessary to exercise care in the amount of moisture furnished, as too much moisture was as hurtful as too little. The results obtained from material properly preserved and from freshly collected material were essentially the same.

The following points are considered: I. Origin and growth of chloragogue; II. Distribution; III. Structure; IV. Function and V. Elimination.

I. *Origin and Growth.*—To learn whether the chloragogue, as is generally supposed, is a modification of the peritoneal epithelium, a series of worms was prepared beginning with a young

[1] "Das Chloragogen von Ophelia radiata" Jenaische Zeitschrift für Naturwissenschaft, 1893, XXVIII., Neue Folge XXI.

worm 5 mm. long and continuing through increasing sizes till a stage was reached in which the chloragogue cells appeared fully developed. In the smallest worms the peritoneal layer, the chloragogue that is to be, is always apparent and a similarity is evident between it and the peritoneal layer elsewhere, both in structure and in reaction to staining fluids. In the smallest worms the chloragogue appears as a simple layer as yet undifferentiated into the club-shaped cells of the adult form. In larger specimens of the series a gradual development into the typical chloragogue cells can be traced. As the cells become older they increase in length, the characteristic granules appear and they become less and less responsive to stains, until in the fully developed adult condition they are practically proof, excepting their nuclei, against every stain to which they were subjected. The nuclei readily respond to stain.

Upon adult worms the following experiment was performed to determine whether there was any renewal of chloragogue when it is artificially removed. With a sharp razor an incision was made through the outer body wall into the cœlomic cavity, exposing the alimentary wall. With a scalpel the chloragogue was then removed from the alimentary wall as completely as possible and the worm afterward kept under favorable conditions of temperature, moisture, etc., in order to insure a condition of thrift. The result of this experiment was that although the wound made in the body wall in order to remove the chloragogue began to heal well the second day after it was made, no chloragogue regeneration was observed in specimens sectioned at various intervals thereafter.

II. *Distribution.*—In the adult worm the chloragogue is found to be distributed as follows: beginning at the posterior end of the œsophagus it extends dorsally, attached in abundance to either side of the dorsal blood vessel as far back as the thirtieth segment (counting from the rear). It is interesting to note that the chloragogue attached to the outside of the alimentary canal *practically* ends in the region where the typhlosole ends: from the thirtieth segment to the anus it becomes less and less abundant, finally disappearing about the seventh somite. In the typhlosole it is also abundant. Somewhat less abundant than

on either side of the dorsal blood vessel, the chloragogue is found surrounding the origin of the aortic arches dorsally, and covering the dorso-intestinal blood vessels, an important fact, inasmuch as the blood gathers from the alimentary wall into the dorso-intestinal blood vessels and through them flows into the dorsal blood vessel. This condition suggests that the chloragogue may with good reason be considered as having to do with food elaboration. The chloragogue is found less abundant still on the outer surface of the alimentary wall, arranged in irregular circular bands. It is also found laterally and ventrally but not as abundantly as on the dorsal aspect. It is entirely absent from the ventral blood vessels, in marked contrast to its abundance on the dorsal vessels. In passing, reference may be made to the fact that within the dorsal blood vessel as seen in cross section a tissue is found, which in appearance, is much like the chloragogue. The largest individual chloragogue cells are found on either side of the dorsal vessel as it crosses the crop and gizzard. The walls of the crop and gizzard are free from chloragogue.

III. *Structure.*—The typical adult chloragogue cell is club-shaped, having at its narrow base a nucleus of medium size which readily stains, while filling the remaining cell space are innumerable minute yellow granules which compose the greater part of the cell and give to it its characteristic color, which varies from a brownish-yellow to a yellowish-green. These chloragogue granules are apparently lifeless. No stain has been found to affect them, the strongest acids like nitric acid and hydrochloric acid affect them but slowly. Likewise strong alkalies like potassium hydroxide have only a very slow effect in disintegrating them. Neither does feeding or starving a worm, as will be shown later, have any apparent effect upon the chloragogue. In the very young worm, however, the entire chloragogue cell is more or less responsive to stains, and the younger the chloragogue the more striking is its likeness to the peritoneal epithelial layer of which it is generally supposed to be a modification.

IV. *Function.*—One of the first attempts to determine the function of the chloragogue was through special feeding. A long series of negative results was obtained. The difficulty was not

found in the worms refusing to eat, because a few hours (over night) sufficed for a worm to gorge itself with whatever substance was placed before it even though it were no more than common white blotting paper. The worm, when the proper amount of moisture and the right limits of temperature were provided, thrived as well on fuchsin-stained earth ; on milk-saturated earth;[1] on finely divided meat scraps ; on moist bread ; on vegetables such as finely chopped leaves of cabbage, lettuce, etc., as it did on its customary earth diet. But no effect due to this varied feeding was observed on the chloragogue. This being so it was determined to try and affect the chloragogue through starving. A number of large thrifty worms were deprived of both food and water for as long a time as they could be kept alive, which was three days. In this time they lost 44 per cent. in weight, but a microscopical study in cross section of a worm thus treated, while it showed a great shrinkage in the cells of other tissues, did not show the chloragogue as having undergone any essential change. It cannot here be said that the function of the chloragogue has been definitely learned ; but the following inferences in the light of the foregoing and what is to follow seem justified. Finding the chloragogue in such close proximity to that part of the blood system which transports the newly absorbed food (see Fig. 1)—dorsal blood vessel, dorso-intestinal blood vessels and aortic arches—and within the typhlosole, and ceasing to be abundant on the outside of the alimentary wall at the point where the typhlosole ends within the alimentary canal, would indicate that the chloragogue had an important relation to the elaboration of food. But the lifeless condition of the adult chloragogue and its apparently living condition in the very young worm, indicate that possibly the function of the chloragogue is performed in the early development of the worm, and that in the adult it has become functionless, although in the

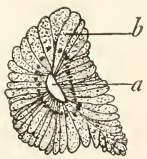

FIG. 1. Showing the characteristic abundance of chloragogue surrounding the dorso-intestinal blood vessels. *a*, dorso-intestinal blood vessel with (*b*) chloragogue cells surrounding it.

[1] Here it may be added that in histological preparations of milk-fed worms, treated with osmic acid, the characteristic black-colored fat globules could be distinguished in the epithelial cells of the alimentary wall but no trace of them was found in the chloragogue cells.

light of what follows the inference seems justified that it may have an excretory function.

V. *Elimination.*—Chloragogue granules are found free in the cœlomic fluid. They are found imbedded in leucocyte bodies. They are found composing the greater part of large waste masses,

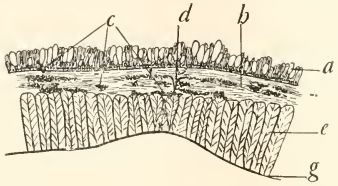

FIG 2. Transverse view of median dorsal section of outer body wall showing characteristic elimination of minute waste masses of chloragogue granules. *a*, epithelial cells (hypodermis); *b*, circular layer of muscles; *c*, minute masses of waste chloragogue granules making their way to the outside dorsally. In Fig. 3 the masses are large but less numerous; *d*, nerve; *e*, longitudinal muscle layer; *g*, peritoneal epithelium of which the chloragogue is a modification.

in some instances filling the entire cœlomic cavity in the posterior region of the body, between numbers of the dissepiments. Lastly they are found in the muscular tissue of the outer body wall, dorsally and ventrally (see Figs. 2 and 3): on the dorsal

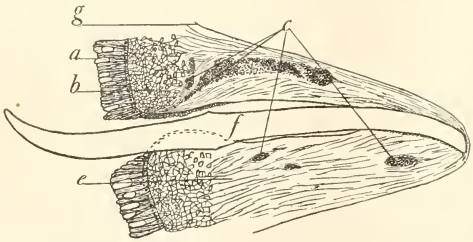

FIG. 3. Longitudinal view of portion of the outer body wall at the level of the ventral row of setæ showing waste elimination ventrally, of waste chloragogue granules in considerable mass. Letters signify same as in Fig. 2 with addition of *f*, seta.

aspect, for the most part as diffuse granular masses distributed throughout the circular muscular layer; on the ventral aspect in more compact masses in the vicinity of the setæ. They appear to be thus making their way to the outside. That there is

in the earthworm such a crude method of waste elimination is further supported by the results of an experiment performed by a fellow student at Lake Forest College, Mr. John J. Jackson. His experiment was undertaken to learn how the earthworm eliminates a foreign substance injected into the cœlomic cavity. Lamp-black was the substance used on this occasion. Histological preparations of material thus treated showed the lamp-black evidently making its way through the tissues of the outer body wall to the outside in the manner described for the waste chloragogue granules. That the letter are thus directly extruded any one may easily demonstrate by the following experiment: A thrifty worm thoroughly washed is placed under a bell-jar and subjected to the influence of ether vapor. The mucus which the worm casts off as a result of this ether vapor irritation, on microscopical examination is seen to contain an abundance of chloragogue granules which are enveloped or embedded in a transparent substance—possibly the contents of a mucous or a leucocyte cell that soon bursts, allowing the granules to scatter in various directions.

In the waste masses, for the most part composed of chloragogue granules, found in the anal region are large numbers of setæ, in one adult worm as many as six hundred and twenty-five (counted in part and estimated) were present, varying in size from small undeveloped to large fully developed ones. The size of the setæ depends somewhat on the size of the worm—the fully developed setæ naturally not being found in a very young worm while in an adult worm both very small and fully developed setæ are often present. Also in many of these same waste masses are found nematodes—*Anguilula lumbricci*, kindly determined for me by Dr. W. M. Woodworth. An interesting question arises in regard to the presence of these nematodes embedded in the waste masses in the posterior region of the body because of the following conditions. While some waste masses are entirely lacking in nematodes, such masses being of a dark brown color, other waste masses again are found which vary from a brown to an almost white color—depending on the number of nematodes present. If they are many, in which case the granules of chloragogue as a rule are scarce, the masses are white. The question

that has arisen from this condition of things is whether there may not be a symbiotic relations between the earthworm and these supposed parasites. Are these nematodes instrumental in consuming the chloragogue waste which the worm by other means is unable to eliminate from its body?

A detailed study of these waste masses both in preserved (for winter use) and freshly collected material gave these results: The average number of setæ (counted in part and estimated) present in the waste masses of each of the seventeen worms examined was two hundred and forty-eight. The nematodes in the dark-colored waste masses were as a rule few—sometimes entirely absent—and, when present, in a quiescent state, often enveloped by a sort of cyst. In these dark-colored masses the chloragogue granules far exceeded the nematodes in abundance.

In the light-colored masses, on the other hand, the nematodes were far in excess of the chloragogue granules, and on pressing the cover-slip many of the nematodes, having within their bodies granules apparently identical as to size and color with those of the surrounding waste chloragogue granules, could be excited to move freely and by so doing clearly showed themselves to be unencumbered by any cyst. Their appearance was suggestive of a newly moulted insect. This supposition of a symbiotic relation is then supported to the extent that within the nematodes have been found granules identical as to size and color with chloragogue granules; and further by the fact that where the nematodes are in excess and in an active condition the chloragogue granules as a rule are far less abundant than the nematodes. It should be stated, however, that the nematodes at no time have been seen in the act of consuming the chloragogue granules, though frequently one has patiently sought to observe this. Still the facts seem to justify the suggestion of a possible symbiotic relation existing between these nematodes and the earthworms within which they live.

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BIOLOGICAL BULLETIN.

ABNORMALITIES IN THE CESTODE MONIEZIA EXPANSA. III.

C. M. CHILD.

In Parts I. and II. of this paper (Child, '00) the various abnormalities occurring in *Moniezia expansa* were described, in Part I. those consisting of imperfect and partial proglottids and in Part II. the spiral abnormalities. It now remains to determine the manner in which these various abnormalities arise, and to discuss the significance of the facts observed.

As is clear from preceding descriptions, there is not the slightest evidence that any of the form-variations of the proglottids appear after development of the proglottid is begun. The degree of differentiation of all parts of an abnormal proglottid is the same, and corresponds to its position in the chain. All the various kinds of form-variations occur in the youngest distinct proglottids. These facts lead to the conclusion that the variations in form appear at the time when the proglottid is formed; that imperfect and partial proglottids and spiral series take their origin in that part of the body in which the causes that lead to the formation of proglottids are at work. That is to say, variations in form are the result of variations in the original formative processes, not the result of modification in the form of structures already established.

Since this is the case, the process of formation of the normal proglottid may be expected to throw some light upon the factors concerned in the formation of abnormalities. The first part of this paper is devoted to a description of the normal method of proglottid-formation.

The figures are all semi-diagrammatic, but in the figures of sections the nuclei of the parenchyma were drawn in with the

camera, and thus correspond as nearly as possible in number and position with those in the actual sections. The subcuticular layer, however, is represented schematically by stippling.

1. Structure of the Neck-Region and Formation of Proglottids.

Behind the scolex there is a region some five to eight millimeters in length, commonly known as the neck, in which no division into proglottids is visible externally.

A few words regarding the structure of the body in this region will render the following description somewhat clearer.

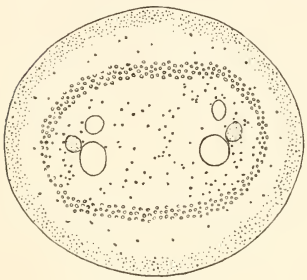


Fig. 42.

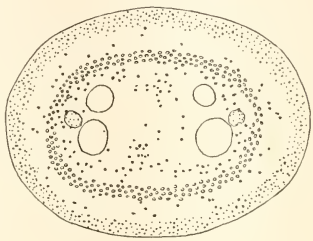


Fig. 43.

Figs. 42 and 43 are transverse sections through the region between the scolex and the point where distinct proglottids appear. Fig. 42 is taken from a section near the scolex, Fig. 43 from one further back. The lines 42 and 43 in Fig. 46 (p. 98) indicate respectively the approximate levels of the two sections. The proglottids are just becoming visible externally at the posterior end of Fig. 46.

The outer cuticular membrane is indicated by the line bounding the figures. Beneath this is a cellular layer, the subcuticular layer, with rather closely packed nuclei, indicated schematically in the figures by the stippled region beneath the cuticle. At this stage of development the nuclei appear to lie in more than one row. Beneath this cellular layer is found the parenchyma. The portion of the parenchyma external to the longitudinal muscles I have, for convenience, designated as the peripheral parenchyma

(Rindenschicht of various authors). This contains a few widely scattered nuclei. The most conspicuous muscle-layer at this stage is the well-developed longitudinal muscle-layer indicated in the figures. Other muscles are not shown. Within the muscle-layer the central region of the proglottid is occupied by parenchyma. This portion of the parenchyma surrounded by the longitudinal muscle layer may be called the central parenchyma (Mittelschicht). In it lie the large ventral and the smaller dorsal nephridial canals and the lateral nerve cords.

In the central parenchyma the nuclei are more abundant than

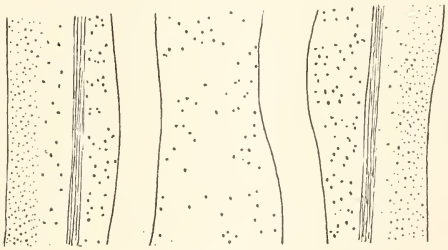


FIG. 44.

in the peripheral layer, and, as the figures show, they are rather more numerous in the outer parts of the parenchyma, especially between the nephridial canals and the muscle-layer.

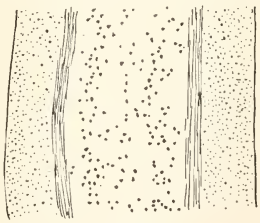


FIG. 45.

The two longitudinal sections, Figs. 44 and 45, show the same structures. Fig. 44 is drawn from a frontal section about half way between the scolex and the first distinct proglottids in the plane of the line 44, Fig. 53 (p. 103). Here the nuclei of the

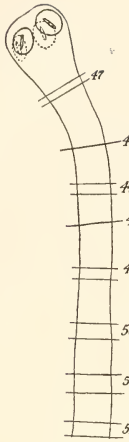


FIG. 46.

central parenchyma are seen to be more abundant at the two sides between the nephridial canals and the muscles. In Fig. 45, which is taken from a sagittal section in the median line (see line 45, Fig. 53, p. 103), the nuclei of this region are seen to be most abundant near the outer boundary of the central parenchyma.

The earliest stages of the growth of the body occur in the neck-region and the proglottids appear at the posterior end of the neck.

Consideration of the method of proglottid-formation requires, therefore a study of the neck-region, and to this the following paragraphs are devoted.

The series, Figs. 47–52, show parts of the neck and the region just posterior to it, viewed as transparent objects. The region of the neck represented by each figure is indicated approximately in Fig. 46 by the numbered areas enclosed by parallel lines, the number in each case corresponding to the number of the figure. All six figures were drawn from a single preparation, a whole mount of scolex and neck-region, which was fixed in the extended condition while slightly flattened between two slides. The longitudinal muscle-layer is indicated by parallel broken lines, and the nephridial canals are drawn in. Nuclei and their general distribution are represented by the stippling.

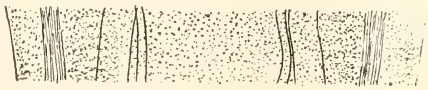


FIG. 47.

In Fig. 47, which shows the region at the posterior end of the scolex itself, the nuclei in the region between the nephridial canals and the longitudinal muscles, where they are most abundant, show faint indications of an arrangement in parallel bands, groups, or zones extending transversely. The groups of the two sides of the body appear to be entirely separate from each other,

the inner end of each band or zone lying in the region of the nephridial tubes. The groups or aggregations of nuclei are so indefinite that it is difficult to count them or to determine whether they correspond on the two sides of the body. The nuclei of the region between the nephridial tubes show no definite grouping.

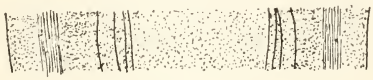


FIG. 48.

Fig. 48, taken further posteriorly (see Fig. 46), shows a similar arrangement of the nuclei within the longitudinal muscle layer.

This peculiar arrangement of nuclei in this region varies somewhat as regards distinctness in different specimens. It can always be made out in preparations which are well extended longitudinally, but in contracted specimens the nuclei are so massed together that it often disappears. The examination of a large number of specimens has, however, satisfied me that it is a normal characteristic of this region.

Fig. 49 is taken from a point near the posterior end of the neck region (see Fig. 46) and shows bands of nuclei extending across the central parenchyma, the first distinct evidences of pro-

FIG. 49.

glottid-formation. The arrangement of the nuclei in these bands is probably due not so much to migration of the nuclei as to more rapid multiplication of certain of them, thus forming aggregations. It may be, however, that migration does occur to some extent. From this point on, these parallel bands of nuclei become more and more distinct and it is clearly evident that they represent the proglottids.

In Fig. 50 an arrangement of nuclei into transverse bands extending across the central parenchyma is still more distinct and

the bands are broader than in Fig. 49. As yet, however, there is no trace of the inter-proglottidal furrows upon the margins or surfaces of the body, and indeed the grouping of the nuclei does not extend into the peripheral parenchyma at all.

FIG. 50.

Fig. 51 shows a still later stage in the process of proglottid-formation. The bands of nuclei are still broader and more distinctly marked in the middle region of the body, though the nuclei are still more abundant in the lateral regions of the central parenchyma. It is evident that the number of nuclei is increasing. One marked advance in the development is noticeable here, viz., the arrangement of the nuclei in the peripheral parenchyma in groups corresponding to those formed within the longitudinal muscles. It is evident that this grouping is gradually extending toward the surface of the body, though it has not yet reached it.

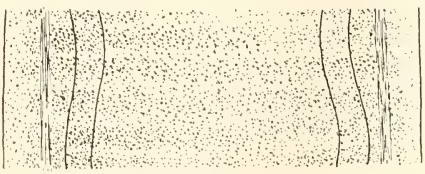


FIG. 51.

In other words, there are in this region of the neck localized regions of growth, or regions in which the nuclei multiply more rapidly than in the intervening, regions and now these areas of more rapid growth are gradually extending toward the surface of the body. All of the figures being drawn to the same scale, it is evident that the growth in width of the body has begun.

In Fig. 52 the proglottids are well defined and their boundaries are indicated upon the surface by shallow furrows. The body is

increasing in width and each proglottid is growing longer as well. Even at this stage the nuclei are seen to be more abundant in the region of the nephridial canals.

The Figs. 49–52 render it sufficiently evident, I think, that each proglottid is simply an area of relatively rapid growth in the parenchyma. The growth first becomes evident in the lateral regions of the central parenchyma, *i. e.*, between the nephridial canals and the longitudinal muscles, where, as was noted above, the nuclei are most abundant. It gradually extends from each side across the middle region of the body, thus forming a zone, which at first includes only the central parenchyma, but gradually extends to the peripheral parenchyma. At last the growth be-

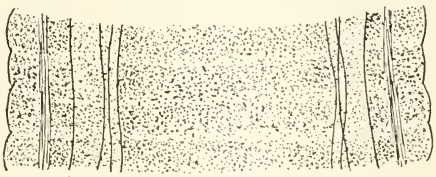


FIG. 52.

comes evident upon the surface, the regions in which it occurs becoming convex as might be expected. At this stage then each proglottid represents a transverse slice of the body which is increasing in size more rapidly than the intervening regions, which therefore become marked as furrows. The furrows are not to be regarded as constrictions, but simply as marking the areas where growth in the transverse direction is least rapid.

The important points in the process are as follows: Each proglottid arises as two distinct aggregations of nuclei, one on either side of the body, which later extend toward the median line and become continuous, and still later extend to the surface or at least to the outer layer of the body.

In Figs. 47 and 48, as noted above, there are traces of a transverse arrangement of the nuclei in the lateral regions of the central parenchyma into bands or groups. The question at once arises as to whether these groups represent the earliest stages of the proglottids, or whether they are connected with some de-

tail of the structure of the neck-region. The evidence on this point is gathered and discussed in the following paragraphs, in which the structure of the neck-region and the early proglottid is examined in detail.

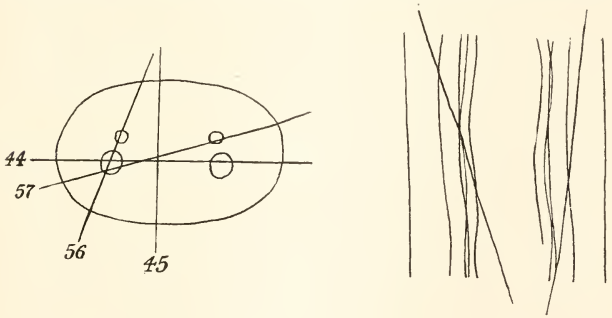
Attention may once more be called to the fact that in the figures of sections the individual nuclei, except those of the subcuticular layer, were drawn in with the camera, so that the arrangement of the nuclei seen in the figures corresponds as closely as possible to their arrangement in the actual section. As the nuclei are distinct and not very numerous, the figures are probably free from serious inaccuracies in this respect.

First of all in this connection, the two transverse sections, Figs. 42 and 43 (p. 96), require renewed consideration. These sections lie in the plane of the aggregation of nuclei and consequently do not show them. Incidentally it may be mentioned, however, that in following through a series of transverse sections from the neck-region, the nuclei between the nephridial canals and the longitudinal muscles are more abundant in some sections than in others and that a certain, not very marked periodical recurrence of the two conditions can be made out. The point of immediate interest shown in Figs. 42 and 43 is the greater abundance of nuclei in the lateral regions of the central parenchyma. In both figures the regions contain numerous nuclei distributed with more or less uniformity, while the region between the two pairs of nephridial canals, though showing some more or less distinct aggregations, contains in general fewer nuclei. This point confirms the evidence afforded by the study of whole mounts, viz., that the nuclei appear to be more abundant in the lateral regions of the central parenchyma than elsewhere when viewed from either surface. Of course in whole mounts the distribution of nuclei in the central parenchyma is more or less marked by the subcuticular peripheral nuclei.

Longitudinal sections are necessary to show the groups of nuclei. The frontal section, Fig. 44 (p. 97), is taken from near the anterior end of the neck. It passes through the ventral nephridial canals. The plane of the section is indicated by the line 44 in Fig. 53. The nuclei of the central parenchyma are seen to be much more abundant lateral to the nephridial canals

than between them. Moreover, an indistinct arrangement in groups of the nuclei of these lateral regions of the central parenchyma does appear, especially on the left side. The groups of nuclei are so illy defined in the preparations in toto, where the whole dorso-ventral thickness of the central parenchyma is seen, that it can scarcely be expected that a section only a few micra (7) in thickness, and thus containing only a small part of the total number of nuclei in a given group, should show the arrangement any more clearly. The nuclei of the peripheral parenchyma are very few in number and scattered, showing no indication of any arrangement in groups corresponding to those of the central parenchyma.

In Fig. 45, a sagittal section near the median plane, the nuclei are seen to be most abundant in two regions, near the outer mar-



FIGS. 53–54.

gins of the central parenchyma, appearing in the section as two longitudinal bands of nuclei, which are in reality sections of two layers of nuclei, a dorsal and a ventral layer. These layers were indicated indistinctly in the transverse sections shown in Figs. 42 and 43. The tendency to aggregation of the nuclei into groups which is present in the lateral regions of the central parenchyma is not visible here, or at least not sufficiently distinct to appear in the section. As regards this point the section confirms the observations on the whole mounts. The region from which this section was taken corresponds approximately to the region from which Fig. 48 was drawn, being probably a little posterior to it.

In Fig. 48 the groups of nuclei, though visible laterally, do not extend to the median plane. It should be noted that the parenchyma-nuclei are more abundant in Fig. 45 than in Fig. 44, thus indicating that multiplication of the nuclei is occurring even in the neck-region anterior to the point where the proglottids be-

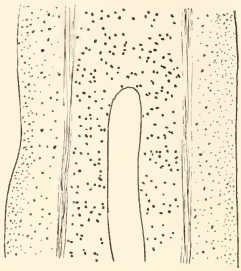


Fig. 55.

come distinct, for Fig. 45 is taken some distance posterior to Fig. 44.

Fig. 55 is drawn from a nearly sagittal section near the posterior end of the neck region. Its plane is indicated by the line

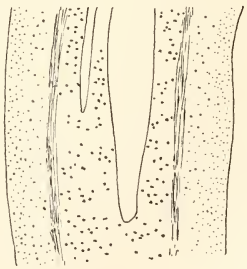


Fig. 56.

55 in Fig. 54. The ventral nephridial canal lies partly in the plane of the section. Here again an aggregation of the nuclei into groups is visible, especially in the lower portion of the figure.

Fig. 56 represents another oblique section which cuts both

nephridial canals. It is taken from about the same region of the neck as Fig. 55, but from another individual. The plane of this figure is represented approximately by the line 56 in Fig. 53. In this figure the grouping of the nuclei is more distinct than in the other longitudinal sections.

These figures represent sections of approximately the same region as is shown in Fig. 49, *i. e.*, a region in which the proglottids first become recognizable. The groups of nuclei represent, therefore, the early stages of what we know to be proglottids, and yet the chief differences between these figures and Fig. 44 consist in the larger number of nuclei and a greater degree of distinctness in the groups of the former figure.

At this point the relation of the lateral groups or zones of nuclei in the anterior neck region to the proglottids requires consideration.

As is evident from the facts stated above, the distinct proglottid extending across the body and marked by a convex contour of the surface appears first at a considerable distance behind the scolex. All through the neck region, however, occur the indistinct groups of nuclei in the lateral regions of the central parenchyma, and, when the proglottids form, their earliest stages appear to arise from the groups.

If these groups represent the earliest stages of proglottid-formation, then the proglottid must be laid down just behind the scolex and pass backward through the neck-region in consequence of the formation of new proglottids in front of it, until finally its development reaches a stage where it becomes visible externally. If the groups of nuclei anterior to the regions where proglottids first become recognizable as bands of nuclei extending over the nephridial canals toward the median plane, do not correspond to proglottids, they must be due to some other features in the structure of the neck. In this case, the neck constitutes an unsegmented region, which gradually becomes marked off into proglottids.

The facts favor the second view. In the first place, the groups of nuclei are just as distinct immediately behind the scolex as they are further back (*cf.* Figs. 47 and 48), *i. e.*, no development occurs in them except perhaps a slight increase in the number of

nuclei until the region is reached where the proglottids become distinguishable. And secondly, I have found similar groups of nuclei in the neck-region of other cestodes, but in a number of cases they did not correspond to proglottids, but a number of them, four or five or perhaps more, were included within a single proglottid when it formed, and a still higher number were found in older proglottids. The examination of *Moniezia expansa* alone might lead to the acceptance of the first of the two alternatives stated above, for when the proglottids do appear they are aggregations of nuclei in the lateral regions of the central parenchyma, of about the same size as the other groups. This comparison with other forms shows that the indistinct grouping of nuclei throughout the neck region has nothing to do with the formation of proglottids. The existence of the groups is probably due to the more or less regular repetition of the dorso-ventral muscles, which are most abundant in the lateral regions of the central parenchyma, and perhaps also in part to the arrangement of the circular muscles. Between the muscles the nuclei of the undifferentiated parenchyma are aggregated into groups. Such an arrangement seems to be visible in some sections, but in others does not appear distinctly.

I have considered this point rather fully because in *Moniezia* the resemblance of the nuclear groups to the early stages of the proglottids is very close.

Returning to the consideration of the forming proglottid, we find that after it becomes visible as a zone of nuclei extending across the central parenchyma, a continuous increase in size in all directions occurs. Some of the features connected with this process of growth require attention here.

In the early stages of the proglottid, before the furrows have become visible on the surface of the body, the parenchyma-nuclei are multiplying very rapidly. Fig. 51 shows the general appearance at this stage, and Fig. 57 is a section of the same stage in the plane of the line 57 in Fig. 53. Here the nuclei of the peripheral parenchyma are just beginning to appear in groups, corresponding in position to the lateral regions of the proglottids in the central parenchyma. Although in whole mounts the boundaries of the proglottids are well marked, sections

show them less clearly. It is interesting to note that the grouping of the nuclei in the peripheral parenchyma appears first in the lateral regions as represented in Fig. 57. Sagittal sections of this stage near the median plane show no grouping of the peripheral nuclei there, although in the central parenchyma the boundaries of the proglottids are quite distinct. Here again the earliest

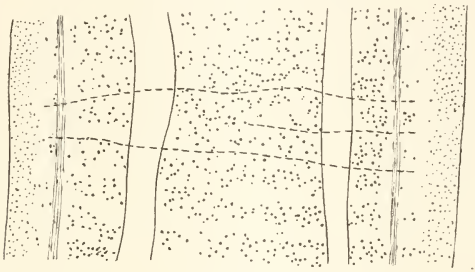


Fig. 57.

stages of development appear near the main nerve-cords and extend gradually to the other parts.

A little later the boundaries of the proglottids become visible on the surface (Fig. 52), and sections at this stage show still further increase in the number of nuclei, especially in the central parenchyma. Fig. 58 represents a sagittal section near the median line. The furrows are beginning to appear on dorsal and ventral surfaces. The outlines of the proglottids are distinct within the central parenchyma and the transverse nephridial canals have appeared. The scarcity of nuclei in the peripheral parenchyma is noticeable, but the few present are more or less distinctly grouped.

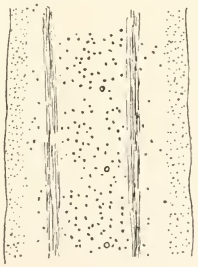


Fig. 58.

A somewhat later stage is represented in the frontal section, Fig. 59. Here the subcuticular cells, which are represented schematically by the dotted region at the margin, have become indistinctly grouped, the spaces between the groups corresponding to the inter-proglottidal furrows. The

peripheral parenchyma-nuclei, though few, are grouped in the proglottids.

Now the proglottid is completely established as a localized region of growth. Later changes which occur lead to some alteration in the shape and proportions of various parts and to the differentiation of various organs within the proglottid.

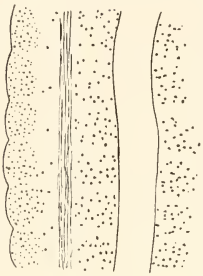


Fig. 59.

In some Cestodes, including *Moniezia*, the longitudinal nerve-cords are connected by a series of transverse proglottidal commissures and other proglottidal nerves are also present. The development of this proglottidal nervous system has been investigated but little, although it is certainly a field of considerable interest. The time at which the nerves appear, their relation to the formation of other parts of the proglottid and themanner of their differentiation are all difficult but important problems.

Tower ('00) has described and figured the origin of the transverse commissures in the neck-region of *Moniezia* (Fig. 1, Taf. 21). Mr. Tower has had the kindness to inform me personally that the folds along the margins of the neck in this figure are not intended to represent the outlines of proglottids but are merely the folds resulting from contraction. He finds that the transverse commissures begin to appear before the proglottids are distinct, but he has not been able to discover any great degree of regularity in the order of their appearance. The first traces of these commissures appear almost immediately behind the scolex, but their formation is not completed until the stage where the proglottids begin to be visible. It is possible, as Tower himself admits, that further study might show a much greater regularity in the development of these commissures.

The point in Tower's figure where the first traces of the commissures appear is certainly far anterior to the region in which the proglottids become distinct, *i. e.*, the commissures are apparently the first of all the proglottidal structures to appear.

The study of the neck-region of *Moniezia* brings to light a

number of facts which possibly indicate a close relation between the appearance and early development of the proglottids and the nervous system. The figures of whole mounts (Figs. 47–52) and of transverse and longitudinal sections through the neck region (Figs. 42, 43, 45) show that the earliest aggregations of the parenchyma-nuclei occur in the region in which the lateral longitudinal nerve-cords lie, and that growth of the proglottids follows the lines which the transverse dorsal and ventral commissures must take as they develop.

In the transverse section, Fig. 43, the nuclei of the central parenchyma are mostly situated in an indistinct ring around the outer margin of the central parenchyma. In Fig. 42, from a more anterior region, this arrangement is less clearly marked. In the longitudinal sagittal section near the median plane (Fig. 45), which is taken from a region posterior to Fig. 43, the same arrangement, or more rapid multiplication of the nuclei is evident in the outer region of the central parenchyma, *i. e.*, in the region which the dorsal and ventral portions of the nervous system occupy.

In general it is evident then that the multiplication of the parenchyma-nuclei occurs most rapidly in regions of the body about the nervous system.

Too little is known regarding the arrangement and development of the proglottidal commissures and nerves in other Cestodes to enable us to determine whether the proglottidal system appears before the proglottids become distinct.

A number of authors have described the nervous system of the proglottids of various cestodes, but little attention seems to have been paid to the region of formation. It seems extremely probable, moreover, that our knowledge of the proglottidal system is incomplete as regards most cestodes. The difficulty of distinguishing with certainty the nervous tissue, not the lack of interest in the subject, is responsible for this condition. Branches arising from the longitudinal nerves have often been mentioned and among the later writers upon the subject a number (Koehler, '94; Scheibel, '95; Lühe, '96; Cohn, '98), have discovered in various forms more or less complex systems of commissures between the longitudinal nerves, sometimes forming a highly developed net-

work. So far as I am aware, the only account of the development of this system is the one given by Tower ('00).

Lühe ('96) and Cohn ('98) have stated that in *Ligula*, where the reproductive organs are repeated but the body shows no segmentation, and in *Schistocephalus*, a segmented form, systems of ring-like and radiating transverse commissures connect the numerous longitudinal nerves. The two sets of commissures always occur together, but are said not to be segmentally arranged. Nothing is known, however, of the development of these commissures, and indeed it is not even certain that other nerves do not exist.

Mr. Tower has very kindly informed me that in abnormal proglottids of *Moniezia* the nervous system was not normally arranged. Unfortunately his notes, drawings, and preparations were destroyed by fire.

It appears then from the little we know of the nervous system of cestodes, and its development in the proglottids, that the system of commissures makes its appearance very early in the history of the proglottid. Tower's account of the nervous system in the neck-region of *Moniezia* renders it very probable that the proglottidal commissures constitute the earliest visible indications of proglottid-formation. As noted above, the nuclei of the central parenchyma in the neck are more abundant in the region of the lateral nerve-cords and in a dorsal and ventral layer which corresponds closely in position to the region occupied by the dorsal and ventral parts of the nervous system. Each proglottid begins to form in two separate parts, one in the region of each lateral nerve-cord; these two parts extend toward the center and unite, *i. e.*, they follow in general the same course of development as do the dorsal and ventral transverse commissures (Tower '00, Fig. 1, Taf. 21). A little later the process of proglottid-formation extends to the peripheral parenchyma; the small nerves extending toward the surface of the body appear, according to Tower, somewhat later than the commissures. Still later in the history of the proglottid the genital organs appear. Tower found certain nerves which he designates as genital nerves, and states that these appear later than the other portions of the nervous system. All of these facts certainly indicate a close connection between the development of the nervous system and that of the proglottid.

It would be premature to attempt with the few facts available to reach a final conclusion with respect to the relations of the two processes. There are three possibilities to be considered: the localization of growth and the formation of proglottids may be the result of the appearance of the transverse nerves and commissures and other parts of the proglottidal system; the development of the nervous system may be the result of the appearance of proglottids; or lastly, both may be effects of a common cause. Which of these three possible views is correct, it is impossible at present to decide. If the first of the three be accepted and it be admitted that some stimulus from the parts of the nervous system as they develop leads to the formation of a proglottid, an explanation is afforded of the first appearance of the proglottid as two groups of nuclei on opposite sides of the body, of the position of these groups, of the direction of their growth and of the order of development of the various parts of the proglottid. It would be out of place here to discuss the influence of the nervous system on growth, differentiation, and regeneration in general, but that such an influence has been found to exist in many cases cannot be denied.

If we accept either of the other two possibilities, no explanation of the observed method of proglottid-formation can be given at present.

The development of the nervous system and the process of proglottid-formation in different cestodes certainly offers a most interesting field for research.

2. The Growth of the Proglottids.

The further history of the proglottid concerns its growth in size, various alterations in shape and the development of the genital organs. It is beyond the scope of this account to enter into

Figs. 60.

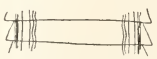


Fig. 61.

the details of the formation of the genital organs, but some features of the growth require consideration.

Figs. 60–63 are outline drawings of the form of the proglottid

at various stages of its history. Fig. 63 shows one half of the proglottid. The longitudinal muscles and the nephridial canals are indicated. Examination of this series will show clearly that

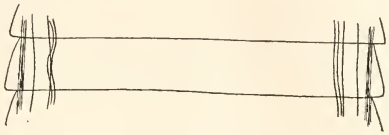


Fig. 62.

almost the whole increase in the width of the proglottid is due to the growth of the central parenchyma, the peripheral parenchyma forming little more than the posterior extensions at the margins.

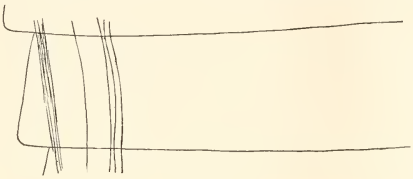


Fig. 63.

In the growth in thickness of the body, *i. e.*, in the dorso-ventral direction, the central parenchyma is not so markedly predominant. The three regions seen in a sagittal section preserve

Fig. 64.

Fig. 65.

Fig. 66.

Fig. 67.

much the same proportions during the growth of the proglottids (Figs. 64–70). In this series Figs. 66 and 67 show respectively the contracted and extended conditions in proglottids of the same age. Figs. 68 and 69 show similar conditions in a later stage. Fig. 70 is from a nearly "ripe" proglottid. In this series it is seen that the peripheral parenchyma has undergone about the

same amount of growth here as at the lateral margins, while the central parenchyma shows very little relative increase in thickness.

In general, the central parenchyma grows chiefly in the lateral and longitudinal directions, the actual increase in thickness being much less than the increase in other dimensions and relatively very slight. The growth of the peripheral parenchyma increases

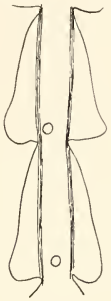


FIG. 68.

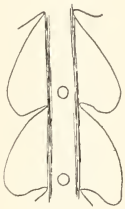


FIG. 69.

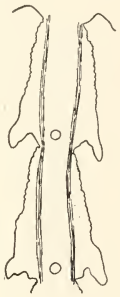


FIG. 70.

in amount from the anterior to the posterior end of each proglottid, but is similar about the whole circumference of the body. The first traces of the posterior extensions of the proglottids are seen in Fig. 71, a sagittal section of an early stage. Nearly all the nuclei of the peripheral parenchyma are grouped near the posterior boundary of each proglottid, indicating that growth is more rapid there than elsewhere, and the outlines of the proglottids are no longer regularly rounded, as in Fig. 59 for instance, but show distinctly localized growth in the posterior region.

The above observations upon the origin and growth of the proglottid may be summed up as follows: The whole neck is a region of growth; in the anterior part of the neck the growth is general but posteriorly it gradually becomes localized and thus gives rise to the proglottids. Each proglottid arises as two distinct aggregations of nuclei in the central parenchyma near the lateral nerve-cord. Gradually the intervening portions of the central parenchyma come to show a similar arrangement and a zone of localized growth is formed. This growth next extends

to the peripheral parenchyma and finally becomes visible on the surface in the rounded contour of the proglottid and the furrows marking it off from others. Later growth, while including all parts of the proglottid, is greatest in amount in the central parenchyma. The relative increase in length and width of the

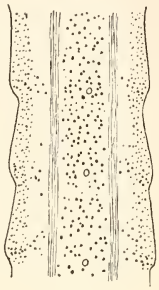


FIG. 71.

proglottid is considerably greater than its increase in thickness. The folds forming the posterior borders of the proglottids are formed by the localized growth of the peripheral parenchyma, and since growth begins here considerably later than in the central parenchyma, these folds are comparatively late in making their appearance.

(*To be continued.*)

SOUTHEASTERN UNITED STATES AS A CENTER OF GEOGRAPHICAL DISTRIBUTION OF FLORA AND FAUNA.

CHAS. C. ADAMS.

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INTRODUCTION.

From a faunal standpoint, southeastern United States, exclusive of Florida, is very remarkable. The fauna is not only very rich in individuals and species, but also in endemic forms. Some of these are indigenous, while others are evidently fragments of an ancient and formerly widespread fauna. Another fact which increases our interest is the general relationship of this fauna to that of the Northeastern United States, both biologically and geographically, since there can be but little doubt that this area, together with the boreal region, contains the most important centers of distribution for Eastern United States since the Glacial Period. Some of these characteristics are likely to escape notice unless we consider the fauna in its entirety, and not as restricted by geographical limits.

Many of the characteristics of this fauna have long been recognized. It has been well established that the temperate fauna and flora of Northern United States were driven *south* before the ice of the Glacial Period, and that with the retreat of the ice to the

north, a northward migration of life took place. I have hoped that the Postglacial centers of dispersal could be more definitely located than simply in the South, and I have found from a study of geographical relationship of the fauna and flora that one important center has been in *Southeastern* United States, with Chattanooga as the approximate center.

Geographical Affinities of the Temperate Fauna and Flora of Eastern United States.

Several years ago my attention was first attracted toward the Southeast in attempting to get some idea as to the probable geographic origin of our Illinois reptilian and amphibian fauna. I was then led to conclude that this fauna was primarily of *southeastern origin.* At the same time I learned that the flora of the upper Mississippi valley came from the Southeast. MacMillan ('92, p. 653) said of the Minnesota valley, with respect to its higher seed plants: "While continentally central, it is by no means botanically central, but is peculiarly an Atlantic coast and southern region." With regard to the species he says (ibid., p. 717): "As a whole the metaspermatic flora of the Minnesota valley presents itself as distinctly eastern and southern by species as before by genera."[1] Dr. Bessey ('92 and '00) has shown that the trees and shrubs of Nebraska came from the Southeast. He says: "A close study of the foregoing facts as to the distribution of our woody plants shows that nearly all have probably migrated to the plains from the east. . . . Nearly all our trees have come up the Missouri bottoms and spread from the southeastern corner of the state west and northwest." The trees of Kansas clearly show the same origin, as is easily seen by comparing Mason's ('92) list of Kansas trees with the one given by Dr. Bessey.

Cope ('96, p. 895) has pointed out the Eastern origin, or center of distribution, of our fish, amphibian, and turtle fauna. Upon closer analysis this fauna will undoubtedly indicate a *southeastern origin.* I have not been able to learn that students of birds and mammals have especially noticed that Southeastern

[1] By reference to a good contour map of the Mississippi valley, it will be easy to see the highway by which this flora reached this region from the Southeast.

United States is a center of dispersal, yet one can hardly doubt that such will prove to be the case, upon investigation. The annual paths of bird migrations favor this idea. One more illustration will be taken from the fauna. Mr. Bryant Walker's ('98, p. 10) study of the Unionid fauna of Michigan clearly shows that this fauna is almost entirely of Mississippi River origin, and the Mississippi River Unionid fauna is well known to be of *southeastern origin*. But it is not necessary to multiply instances, because the same law holds for the bulk of our fauna and flora and will be recognized by one familiar with any fairly large group of animals. The affinities of both fauna and flora thus point to, and converge toward the Southeast. About the only marked *exceptions* to the rule are the distinctly *northern* or *boreal* groups.

Such illustrations as the foregoing are significant and sufficient to show that to the Southeast is a region deserving of special attention from a faunal standpoint. Because it is believed that here is the natural starting point for an attempt to untangle many faunal and floral problems, evidence has been collected which seems to throw light upon some of its peculiarities.

Richness, or Abundance and Diversity of Life.

As an illustration of the abundance of life, I will consider the flora, which shows a luxuriant development. Sargent ('84, p. 4) says: "Upon the slopes of the southern Allegheny Mountains, and in the valley of the lower Red River, regions of copious rainfall and rich soil, the deciduous forest of the continent attains unsurpassed variety and richness."

The richness of the fauna is shown by both the abundance and size of the individuals. Binney ('85, p. 34) has called attention to this in the case of the land shells of the "Cumberland Subregion" of the southern Appalachians. He says: "If to the 39 species catalogued above as peculiar to this subregion, are added the 69 which inhabit it as a portion of the interior region, it is seen that in the Cumberland subregion we find the largest number of species of any portion of North America. The subregion is equally prolific in individuals, and the individuals are highly developed."

The fresh water molluscan fauna is even richer than the land

shell fauna. Mr. Charles T. Simpson, of the U. S. National Museum, has told me that within 200 or 300 miles of Chattanooga there are more valid species of Unionidæ than in all the rest of the world. As to the Pleuroceridæ, about 300 or 400 species are found in this same area. These and the Viviparidæ here reach their largest size. Here also is the maximum differentiation of the crayfish. The states of Georgia, Alabama and Tennessee, have, according to Faxon ('85, p. 179), the richest crayfish fauna—36 species.

The vertebrate fauna is also very rich. Jordan ('96, p. 114) says of the fish fauna: "These conditions [favorable for fish] are all well realized in the Washita River of Arkansas, in the various tributaries of the Tennessee, Cumberland, and Ohio, and in these streams, among the American streams, the greatest number of species have been recorded."

Endemism and Relicts in the Fauna.

There is still another line of evidence which indicates that this region is not only a recent center of dispersal, but in addition, an area of preservation of ancient types. While it is in this region that the indigenous fauna of Eastern United States reaches its greatest development, it is not these forms primarily to which I wish to call attention now, but to the *relicts* or fragments of a still more ancient fauna. For convenience these will be discussed in two groups:

1. *Relicts having Asiatic Affinities.*—Years ago Asa Gray ('78), and Hooker ('79), and more recently Sargent ('94), have discussed the close relationship existing between many plants found in Eastern America and others found in Japan or Eastern Asia. This discontinuous distribution of many of these forms indicates that they are fragments of an ancient flora. Hooker says: "This generic identity, however, gives but a faint idea of the close relationship between East American and East Asiatic, especially of the Japanese floras, for there is further specific identity in about 230 cases, and very close representation in upwards of 350; and what is most curious is that there are not a few very singular genera of which only two species are known, one from east Asia, the other in east America." The tulip tree (*Liriodendron*), bald

cypress (*Taxodium*), sweet gum (*Liquidambar*), and tupelo or sour gum (*Nyssa*) are interesting examples of genera limited to Eastern Asia and Eastern North America.

But little attention has been given to the Asiatic relationship of the fauna of Eastern United States. No case has come to my notice of a mammal or bird which is limited to *southeastern* United States and shows close East Asiatic affinities. The rodent genus *Zapus* is solely American excepting a single species in Western China (Miller, '00, p. 112). The isolated East Asiatic relative (*Sialia cœlicolor* Hods.) of our common blue bird (*Sialia sialis* L.) is interesting in this connection (Seebohm, '81, p. 328). But our alligator has a near relative in China. The salamander fauna (Gadow, '01, p. 95) of Eastern North America is the richest in the world. *Triton*, as in the case of the dragon-fly *Boyeria*, mentioned below, occurs in Eastern United States, Europe and Eastern Asia. The genus *Amblystoma* is abundant in Eastern North America and has only a single species in Eastern Asia. But the most interesting case among the salamanders is that of the hellbender (*Cryptobranchus alleghaniensis*) whose only near relative is the giant salamandar (*C. japonicus*) of Japan, which attains a length of over five feet. Among fish, Cope ('75, p. 64) gives three genera, the spoon-bill (*Polyodon*), the carpsucker (*Carpiodes*) and a catfish (*Ameiurus*), each represented in China with one species; and Jordan ('96, p. 100) gives the shovel-nosed sturgeon (*Scaphirhynchus*) with relatives in Central Asia.

Among crustacea, the occurrence of *Limulus* upon the Atlantic coast with relatives in Asiatic waters and the freshwater *Peneus* in Eastern United States and also at the foot of the Himalayas (Semper, '81, p. 437), are to the point. Huxley, in his "Crayfish" (p. 334), has pointed out a similar relation among crayfish. Faxon ('85, p. 176) says, concerning this relationship: "The Cambari of Eastern United States are mostly related, not to the crayfishes on the other side of the Rocky Mountains, nor to those on the opposite shore of the Atlantic, but to those of the remotest district, Eastern Asia."

Among insects we have some interesting relicts. For example, we have a dragon-fly (*Hagenius brevistylus*) with its nearest

relative in Japan, and also *Boyeria*, with three species, one species each in Japan, United States and Europe. Our luna moth (*Tropæa luna*) has a close relative in China (*selene* Leach). The Bryozoan genus *Pectinatella*, although mainly of Northern distribution in the United States, has two species, one in Eastern United States, and the other (*P. gelatinosa*) in Japan.

This oriental relationship has been clearly pointed out with regard to some of our Unionidæ by Simpson ('96, p. 330). He says: "The Naiad fauna of this region [Oriental] is magnificent and diversified, and almost rivals that of the Mississippi Valley in vigor, size, solidity and variety of forms. Both Dr. C. A. White and von Ihering believe that the Unios and Anodontas of this area are closely related to those of the central part of North America. Not only does there seem to be a general relationship among a large number of the Naiads of this province with those of the Mississippi basin, but several oriental groups are apparently so close to those of our own region that it is well-nigh impossible to separate them. Thus, the Asiatic Anodontas, typified by *A. woodiana* Lea if found in the United States, would be placed by most students with *A. plana;* the Chinese Unios of the group of *U. housei* Lea, and *myersianus* Lea, are evidently quite near the *Alatus* assemblage; *Unio superbus* Lea, is very much like our *U. capax* Green, and a number of the tuberculate forms of China could almost be placed in the American groups of *U. lachrymosus* and *U. pustulosus*." As has been said before, it is well known that the Unionidæ fauna of the Mississippi River system has been derived from that of Southeastern United States. It is important to notice that the East Asiatic affinities are so distinctly marked in the case of the *fresh water fauna.*

The same explanation which Asa Gray gave for the flora, doubtless holds for the fauna. In Preglacial times the flora had an extensive boreal distribution, as shown by Greenland fossils; and with the advance of the ice, life was driven South in both hemispheres, and has been preserved in both, in favorable localities. An analogous case in the other hemisphere is the preservation of certain mammals in Africa and in the Oriental region (Osborn, '00, p. 57) and Unionidæ (Simpson, '96, p. 340).

Relicts not Showing Asiatic Affinities.

Among animals, there are not only members of this fauna showing close Asiatic affinities, but there are other relicts which do not show such a relationship. Jordan ('88, p. 168) has given a number of genera of lowland fish which he considers archaic forms, or members of an ancient fauna. The mud minnow (*Umbra*), the pirate perch (*Aphredoderus*), the pygmy sunfishes (*Elassoma*), and the blind fishes (Amblyopsidæ), are examples of such forms.[1] Among butterflies *Libythea* is of this type also, and *Tachopteryx* among dragon-flies, and doubtless many other insects are.

Two Centers of Dispersal in Southern United States.

That the South was a retreat for life during the Ice Age is too well known to be discussed here. On account of the great amount of endemism and relicts in the Southeast it seems fair to conclude that quite an abundant and diversified fauna and flora lived there during the Ice Age, although the contrary opinion is often expressed. In south temperate North America there seem to be two very distinct and powerful centers of dispersal, or centers of "adaptive radiation," as Osborn ('00, p. 49) might call them. One, to which we have been calling attention, is in the Southeast, and the other in the Southwest on the arid plateau of Mexico and Southwestern United States. This latter area, or distinct center of dispersal, has a very large number of indigenous forms, as is shown by Merriam's system of zoögeographical areas ('93, p. 401). This is undoubtedly the area where the characteristic arid fauna (and flora)[1] of North America has originated or has had its center of dispersal. The great amount of endemism in this area has suggested that this may have retained much of its present arid condition for a considerable period of time, possibly preceding and during the Ice Age; and one condition of its present richness may be due to its greater distance from the ice-sheet which destroyed many forms in Southeastern United States. The heavy rainfall and forests of Southeastern United States should be contrasted with the arid plains and deserts of the Southwest and the corresponding difference in the types of animals originating in these two regions, noted.

[1] Gray, '81, p. 62, and Bray, '00, p. 713.

Northeastern United States during the Ice Age evidently could have had only a boreal fauna and flora. The present distribution of the boreal fauna and flora is along the northern border of the United States and the higher parts of the Appalachian Mountains. But it is not this element with which we are primarily concerned, because we have seen that the biogeographical affinities of the forms which at present dominate in the Northern United States east of the Great Plains do not point to a boreal origin, nor to the arid region of the plains, nor to the Southwest, but to the *Southeast.*

The criteria, which have been used to determine this center of dispersal will next be considered.

Criteria for the Determination of Centers of Dispersal.

In attempting to determine centers of dispersal of animals, I have found it necessary to keep clearly in mind the tests which are legitimate for such determinations. In a very large number of cases we do not have paleontological evidence in sufficient abundance to materially aid us. For this reason I have thought it desirable to state the criteria used.

1. Location of greatest differentiation of a type.
2. Location of dominance or great abundance of individuals.
3. Location of synthetic or closely related forms (Allen).
4. Location of maximum size of individuals (Ridgway-Allen).
5. Location of greatest productiveness and its relative stability, in crops (Hyde).
6. Continuity and convergence of lines of dispersal.
7. Location of least dependence upon a restricted habitat.
8. Continuity and directness of individual variations or modifications radiating from the center of origin along the highways of dispersal.
9. Direction indicated by biogeographical affinities.
10. Direction indicated by the annual migration routes, in birds (Palmén).

Some of these tests have long been utilized, but, so far as I have been able to learn, all have not been definitely used for this particular purpose. The fifth (productiveness) is very closely related to the second (dominance). It is very well known that

often plants and animals taken from their indigenous country to a new one, require a very favorable habitat in order to live. Hollick ('93, p. 196) and Cowles ('01, p. 105) have called attention to certain plants of more general distribution in the South, which in the North occur only under very favorable or restricted conditions. This change of habit and habitat with divergence from the center of dispersal is a subject worthy of special attention. If Osborn's law of adaptive radiation is of general application, we should expect just such a change in habitat as has been noticed to accompany the divergence from the original home.

The eighth deserves some special attention on account of the present active interest taken in the study of individual variation, to which reference will be made later. The direction of biogeographical affinities and convergence of lines of dispersal, as has been shown, first called my attention to the Southeast.

Outlets or Highways of Dispersal from the Southeast.

Next in importance to the centers of origin themselves are the highways from and to these regions. From the Southeast we have several very well-marked outlets, as follows:

1. The Mississippi Valley, and valleys of tributary streams: the leading outlet.

2. The Coastal plain, leading along the Atlantic seaboard northward, and along the gulf coast to Florida, and the Southwest.

3. The southern Appalachians and adjacent plateaus, forming an outlet to the North.

These are the principal paths of dispersal; two are lowland, one is upland. The lowland highways have functioned not only for the Southeast, but also for the fauna of tropical origin, as Webster ('98, p. 72) has shown to be the case for the chinch-bug, which has pushed into the United States via Florida and the West Indies and also from Mexico. Of course, these outlets may serve also as inlets from other regions.

Aquatic life has been greatly favored by the Mississippi River system. From the Southeast, the Tennessee River has undoubtedly been the leading outlet; and next, perhaps, the Cumberland River. Formerly the ancient Appalachian River (*i. e.*, the Tennessee above Chattanooga plus the Coosa-Alabama River)

in the Appalachian Valley, was an important highway for aquatic life, and the valley remains to the present time a highway for land forms.

Desirability of Studying the Variations or Modifications in Animals and Plants as They Diverge From Their Center of Origin Along Their Lines of Dispersal.

With the increased interest which is now being shown in the study of individual variations of plants and animals, it is of value to see the relation which such studies have to the highways of dispersal, and vice versa. The real importance of this relation does not seem to be fully realized. This fact is apparent when the geographical variations are largely expressed in terms of the cardinal points, instead of in their relation or deviation from the center of dispersal or changed environment. On account of the topographical relations and past climatic changes of our country, most of the migrations have been deflected more or less to the North or South, and thus such expressions apply in general.

A few points will be mentioned to which special attention is directed:

1. Variation in size, its diminution from the central area. Allen ('76, p. 310) has shown that in North America birds and mammals of austral origin tend to increase in size to the South, while those of boreal origin tend to decrease in size. How general the application of this law is among other animals, has yet to be determined, nor is it known how much the paths of dispersal may influence this. This seems to show concentric divergence from the center of origin. Allen ('71, p. 92) has also called attention to important variations in the size of "peripheral organs" in birds, such as the tail, beak and claws. Weed ('92 and '93) has published two interesting papers on the geographical variations of the harvest-spiders, which show that they increase in size of body and length of legs to the South.

Jordan ('93, p. 23), in speaking of the increase in the number of vertebræ in fish toward the North, says: "In most cases, as the number of vertebræ increases, the body becomes proportionally elongate. As a result of this, the fishes of the Arctic waters are, for the most part, long and slender, and not a few of

them approach the form of eels. In the tropics, however, while elongate fishes are common enough, most of them (always excepting the eels) have the normal number of vertebræ, the greater length being due to the elongation of their individual vertebræ, and not to their increase in number." The relation of these changes to paths of dispersal appears to be close, in some cases at least, as Jordan ('01, p. 566) emphasizes that there is a North and South trend of the lines of distribution of shore fishes.

2. Very closely related to the above law of size and that of dominance of a type in its native home is another formulated by Hyde ('96, p. 575) of the U. S. Department of Agriculture, concerning the productiveness of crops. He says: "Entirely independently of whether the average yield per acre be high or low, the nearer the approach to the region to which a product is indigenous, the more uniform will be the rate of production from year to year; and the farther the departure from such region the greater the liability to fluctuation. This law clearly indicates the greater stability in the indigenous area, and increased variability, with departure from that center. The close relation of the law of size and Hyde's law of productiveness indicates a general tendency which is surely of considerable economic as well as scientific importance, especially with regard to the introduction and acclimatization of foreign plants and animals.

3. The continuity and directness of the individual variations along diverging lines of dispersal is of considerable importance. This fact was impressed upon me by the study of a very extensive collection of gasteropod shells from many localities in the Tennessee River system.[1] There are two very distinct types of shells, which intergrade more or less, in a progressive way, as one passes down stream from the head-waters; one extreme type being in the head-waters and the other farther down stream. It is the degree of continuity or progressiveness in the deviation of the characters which clearly points in definite directions, and emphasizes the necessity of a study of series taken along the lines of dispersal.

In the case of the land fauna the situation is much more complex, yet if highways of dispersal are considered analogous to

[1] Proc. A. A. A. S., 1900.

streams, we are furnished with a good working hypothesis, since the convergence of these lines points to a center of dispersal.

4. The study of variation is essentially a study in biological dynamics, because the lack of stability is the very characteristic considered. The close relation between centers of origin and highways of dispersal emphasizes the desirability of considering faunal and floral areas from the same dynamic standpoint. By this method we can understand the relationships and form of these areas much better. Such areas are *dynamic centers* from which tension lines and zones radiate; and the genetic relationships of these areas can only be determined by a study of the present and past dynamic relations. An important step was made in this direction when Gray and Merriam (Merriam, '90, p. 24) recognized that our North American flora and fauna are composed of *two elements*, boreal and austral, each tending to move from its center of origin.

From a dynamic and genetic standpoint, the limits and significance of life areas take on a new meaning and importance when, as it were, we stand at the center or focus of dispersal, and look outward. From this point of view we look upon the life of a region as constantly diverging or radiating from its original home and the parental stock, encountering new conditions of environment and becoming modified in both habits and structure. The continuity in variations, which is one of the most marked characteristics in the geographical variations, can be safely determined only by the study of the form along its highways. The paths of dispersal are the lines along which we may expect the hereditary factors to show their influence most distinctly. Again, these paths bear the same relation to each other as the branches of a phylogenetic tree, and may therefore be compared with such branches. In phylogenetic studies the most fruitful results do not come from a comparison of the tips of the different branches but from a study and comparison of forms along the same branch and therefore of common descent.

We may with advantage carry this comparison still further, and consider the genetic relationships which faunal areas bear to each other. The lines of kinship are shown primarily along the lines of dispersal. Here, as before, it is not to the relationships which

exist between the terminal branches on which we are to focus our attention, but to those affinities which exist along the highways toward the center of origin. The double origin of the North American fauna and flora will illustrate the present standpoint. The lines of dispersal may be represented by two trees, one with its roots in the North, and the other with its roots in the South, and whose branches intermingle. From these two centers, life has radiated in all possible directions, but on account of the North and South trend of the topography the lines of dispersal have, in the main, been North and South. For this reason the general lines of kinship are likely to be most strongly expressed in these same directions.

It is a very fundamental law that most forms of life are confined to restricted areas and only a small number have extensive distribution. Thus, from the centers of origin there is a constant decrease, or *attenuation* in the number of forms which have been able to depart far from the original home. From this standpoint, therefore, the question arises as to what criteria are to be used in the determination of life areas. Should more stress be laid upon the concentric relations, or degree of attenuation, from the center of dispersal, or, on the other hand, upon the genetic affinities, such as exist along the lines of dispersal? Are the natural affinities better expressed zonally or dendritically? In one case the tree is horizontally truncated and in the other the branches are torn apart.

Surely the genetic and dynamic standpoint, which attempts to explain the life of an area in terms of its origin, favors, as a working hypothesis, a trial of the dendritic method. The method of work here suggested will put faunal studies upon a *distinctly genetic basis.*

From the relationship which exists along the lines of dispersal, we draw an important practical conclusion. In collecting a series of animals for the study of variation, one of the first things to be done is to determine its means and highways of dispersal and then secure material from along these lines. This will greatly simplify the study of land forms, because in this manner we have a clue to determine which localities are likely to be the most important, and upon these we may concentrate our attention.

5. The geographical distribution of color variations is a subject which it is very desirable to study from the standpoint of geographic origin as well as from that of climatic influences. Allen ('71, '72, '74, '77 and '92), especially, has given much attention to the formulation of the laws of geographic color variation among North American birds and mammals, and correlates some of these variations with that of moisture. Ridgway ('72) has also an important paper on color variations in birds. Keeler ('93) and Hasbrouk ('93) are referred to for further information on color variations. These papers give numerous cases showing that species of wide distribution change in color in different localities. These papers cannot be discussed here, but deserve to be much better known.

Summary.

First. In general the fauna and flora of Northern United States East of the Great Plains are geographically related to those of the Southeast and this geographical relationship points to an origin in the direction of the Southeast except in the case of the distinctly boreal forms.

Second. The abundance and diversity of life in the Southeast indicates that it has been, and now is, a center of dispersal.

Third. The relicts indicate that the Southeast has been a center of preservation of ancient types, and the endemism shows that it has been a center of origin of types.

Fourth. There are two distinct Southern centers of dispersal in temperate United States; one in the moist Southeast, and the other in the arid Southwest.

Fifth. Ten criteria, aside from fossil evidence, are recognized for determining the center of origin or the locality of dispersal:

1. Location of the greatest differentiation of a type.
2. Location of dominance or great abundance of individuals.
3. Location of synthetic or closely related forms. (Allen.)
4. Location of maximum size of individuals. (Ridgway, Allen.)
5. Location of greatest productiveness and its stability, in crops. (Hyde.)
6. Continuity and convergence of lines of dispersal.

7. Location of least dependence upon a restricted habitat.

8. Continuity and directness of individual variations or modifications radiating from the center of origin along the highways of dispersal.

9. Direction indicated by biogeographical affinities.

10. Direction indicated by annual migration in birds. (Palmén.)

Sixth. There are three primary outlets of dispersal from the Southeast:

1. The Mississippi Valley and its tributaries.

2. The Coastal Plain.

3. The Appalachian Mountains and adjacent plateaus.

The first two have also functioned for tropical types, and the third for boreal forms. Dispersal is both forward and backward along these highways.

Seventh. The individual variations of animals and plants, such as size, productiveness, continuity of variation, color variation, and change of habit and habitats, should be studied along their lines of dispersal and divergence from their center of origin. Life areas should be studied as centers of dispersal and origin, and hence *dynamically* and *genetically*.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO, December, 1901.

REFERENCES.

Allen, J. A.

'71 On the Mammals and Winter Birds of East Florida, with an Examination of Certain Assumed Specific Characters in Birds, and a Sketch of the Bird Faunæ of Eastern North America. Bull. Mus. Comp. Zool., Vol. II., No. 3, pp. 161–450.

'72 Geographical Variation in North American Birds. Proc. Bost. Soc. Nat. Hist., Vol. XV., pp. 212–219.

'74 On Geographical Variation in Color Among North American Squirrels; with a list of species and varieties of American Sciuridæ occurring north of Mexico. Proc. Bost. Soc. Nat. Hist., Vol. XVI., pp. 276–294.

'76 Geographical Variation Among North American Mammals, especially in respect to size. Bull. U. S. Geol. and Geogr. Survey of the Terr. (Hayden), Vol. II., No. 4, pp. 309–344.

'77 The Influence of Physical Conditions on the Genesis of Species. Radical Review, Vol. I., pp. 108–140.

'92 Variations in Vertebrated Animals. Amer. Nat., Vol. XXVI., pp. 86–89.

Bessey, C. E.

'92 A Preliminary Report on the Native Trees and Shrubs of Nebraska. Bull. Agric. Exp. Stat. of Neb., Vol. IV., No. 4, pp. 1–32.

Bessey, C. E.

'00 The Forests and Forest Trees of Nebraska. Ann. Rep. Neb. State Bd. of Agric. for 1899, pp. 79–102.

Binney, W. G.

'85 A Manual of American Land Shells. Bull. 28, U. S. Nat. Mus.

Bray, W. L.

'00 The Relations of the North American Flora to that of South America. Science, N. S., Vol. XII., No. 306, pp. 709–716.

Cope, E. D.

'75 On Geographical Distribution of the Vertebrata of the Regnum Nearcticum, with especial reference to Batrachia and Reptilia. Bull. U. S. Nat. Mus., No. 1, Pt. III., pp. 55–95.

'96 The Geographical Distribution of Batrachia and Reptilia in North America. Amer. Nat., Vol. XXX., pp. 886–902, 1103–1026.

Cowles, H. C.

'01 The Physiographic Ecology of Chicago and Vicinity; a study of the origin, development and classification of plant societies. Bot. Gaz., Vol. XXXI., pp. 73–108, 145–182.

Faxon, W.

'85 A Revision of the Astacidæ. Memoirs Mus. Comp. Zool., Vol. X., No. 4.

Gadow, H.

'01 Amphibia and Reptiles. Cambridge Natural History, Vol. VIII., New York.

Gray, Asa.

'78 Forest Geography and Archæology. Amer. Journ. Sci. and Arts (3), Vol. XVI., pp. 85–94, 183–196.

Hasbrouk, E. M.

'93 Evolution and Dichromatism in the Genus Megascops. Amer. Nat., Vol. XXVII., pp. 521–533, 638–649, pls. I.–V.

Hollick, Arthur.

'93 Plant Distribution as a Factor in the Interpretation of Geological Phenomena, with Special Reference to Long Island and Vicinity. Trans. N. Y. Acad. Sci., Vol. XII., pp. 189–202.

Hooker, J. B.

'79 The Distribution of North American Flora. Amer. Nat., Vol. XIII., pp. 155–170.

Hyde, John.

'98 Variations in the Rate of Agricultural Production and One of its Causes. Science, N. S., Vol. VIII., No. 200, Oct. 28, pp. 575–576.

Jordan, D. S.

'88 Report of Explorations made during the Summer and Autumn of 1888, in the Allegheny Region of Virginia, North Carolina, and Tennessee, and Western Indiana, with an Account of the Fishes found in each of the River Basins of Those Regions. Bull. U. S. Fish Com., Vol. VIII., pp. 97–173.

'93 Temperature and Vertebræ—A Study in Evolution. Wilder Quarter-Century Book, Ithaca, N. Y., pp. 13–36.

'96 Science Sketches. New edition, Chicago.

'01 The Fish Fauna of Japan, with observations on the Geographical Distribution of Fishes. Science, N. S., Vol. XIV., No. 354, pp. 545–567.

Keeler, C. A.

'93 Evolution of the Colors of North American Land Birds. Occas. Papers Cal. Acad. Sci., Vol. 3.

MacMillan, C.

'92 The Higher Seed Plants of the Minnesota Valley. Geol. and Nat. Hist. Surv. Minn., Botanical Series, 1, Minneapolis.

Mason, S. C.

'92 A Preliminary Report upon the Variety and Distribution of Kansas Trees. Eighth Biennial Rep., Ks. State Bd. Agric., 1891–92, pp. 259–274.

Merriam, C. H.

'90 Results of a Biological Survey of the San Francisco Mountain Region and Desert of the Little Colorado, Arizona. N. Amer. Fauna, No. 3, U. S. Dept. of Agric.

'93 The Geographical Distribution of Life in North America, with special Reference to the Mammalia. Smithsonian Rep., 1891, pp. 365–415. (Also Proc. Biolog. Soc. Washington, Vol. VII., pp. 1–64.)

Miller, Jr., G. S.

'00 Key to the Land Mammals of Northeastern North America. Bull. N. Y. State Mus., Vol. 8, No. 38.

Osborn, H. F.

'00 Correlation between Tertiary Mammal Horizons of Europe and America. Ann. N. Y. Acad. Sci., Vol. XIII., No. 1, pp. 1–72.

Ridgway, R.

'72 On the Relation between Color and Geographical Distribution in Birds, as exhibited in Melanism and Hyperchromism. Amer. Journ. Sci. and Arts (3), IV., pp. 454–460; V., pp. 39–44.

Sargent, C. S.

'84 Forests of North America. Tenth Census of the U. S., 1880, Vol. IX., Washington.

'84 Forest Flora of Japan. Boston. (Also Bessey, Amer. Nat., Vol. XXIX pp. 1049–1056.)

Seebohm, H.

'81 Catalogue of the Birds in the British Museum, Vol. V. London.

Semper, K.

'81 Animal Life. N. Y.

Simpson, Chas. T.

'96 The Classification and Geographical Distribution of the Pearly Fresh-Water Mussels. Proc. U. S. Nat. Mus., Vol. XVIII., No. 1068, pp. 295–343, pl. IX.

Walker, Bryant.

'98 The Distribution of the Unionidæ in Michigan. 1898.

Webster, F. M.

'98 The Chinch Bug: its Probable Origin and Diffusion, its Habits and Development, Natural Checks and Remedial and Preventive Measures, with Mention of the Habits of an Allied European Species. Bull. 15, N. S., Div. of Entomology, U. S. Dept. of Agriculture.

Weed, C. M.

'92 The Striped Harvest Spider: A Study in Variation. Amer. Nat., Vol. XXVI., pp. 999–1008, pls. XXVII.–XXIX.

'93 The Cinnamon Harvest Spider and its Variations. Amer. Nat., Vol. XXVII., pp. 534–541.

THE INTERNAL INFLUENCES THAT DETERMINE THE RELATIVE SIZE OF DOUBLE STRUCTURES IN PLANARIA LUGUBRIS.

T. H. MORGAN.

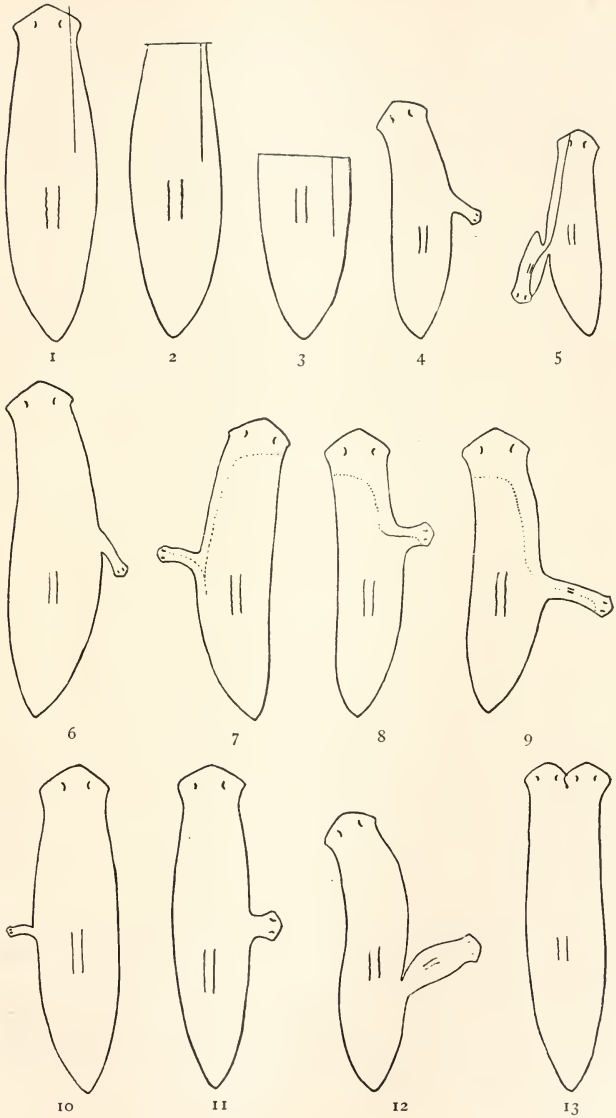
In a previous paper[1] I attempted to determine what internal factors regulate in planarians the limit of size of each of two heads when such are present. Two conditions appeared to have an influence on the result: (1) The width of the region connecting the part to the rest of the organism, (2) the length of the new part. By the following experiments I have attempted to gain the further insight into the conditions:

In one series the planarians were split lengthwise into unequal parts, as shown in Fig. 1; or else the head was first cut off, and then the posterior piece was split lengthwise, as shown in Fig. 2. Under both of these conditions the head on the smaller piece is much smaller than that on the larger part. The purpose of the experiment was to see if by abundant feeding the size of the smaller head could be brought up to that of the larger; or whether its size is determined by the width of the region connecting the small head with the larger piece. If the longitudinal cut does not extend as far posteriorly as the old pharynx, a new pharynx does not, as a rule, come into the smaller part. If, however, the cut is extended posteriorly as far as, or beyond, the old pharynx, a new one may come into the small part. The same end can be reached by cutting off the worm in front of the pharynx as shown in Fig. 3, and then splitting the posterior piece lengthwise. The side piece will be, of course, shorter in the last case.

The results show that the size that the new smaller head attains is determined largely by the presence or absence of a new pharynx in the small part.

The following cases will serve to illustrate some of the ways in which the regeneration takes place. A narrow piece was split off in one case as shown in Fig. 1. A small head developed at

[1] *Roux's Archiv*, XIII., 1901.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its anterior end, but there was no pharynx present. The small piece was attached to the main body at the side and just in front of the anterior end of the pharynx, Fig. 4. Five months later it had not grown any larger (although the worm was kept well fed), but, in fact, appeared to be smaller than at first, Fig. 6. In two other cases the split had extended so far back that a pharynx developed in the small piece, Fig. 5. A tail also grew out at the new side behind the new pharynx. Subsequently the two pieces pulled apart. In another series the old head was cut off by a cross-cut, and then a split made down one side, right or left, Fig. 2. The condition of the two heads a month and a half later is shown in Figs. 7, 8, 9. In the first and second cases a pharynx is absent, but in the third a pharynx has developed in the middle of the smaller worm. The latter condition of these three worms, five months after the operation, is shown in Figs. 10, 11, 12. In the first the head at the side is smaller than it was before ; in the second it has remained about the same size ; while in the third, which contains a pharynx in the smaller piece, the head and the piece as a whole have increased in size.

In another series the heads were split exactly in the middle line. In some of these the split was made only in the anterior end—the cut not extending posteriorly to the pharynx—in others the cut extended into the pharynx region. In other cases the head was first cut off and then the worm split in the middle line. When the head was split for only a short distance, each half completed itself—if the parts were kept from reuniting—as shown in Fig. 13. The head remained smaller in size than the normal head, and even after seven months had not increased any further in size. The inner sides of the heads were a little smaller in size than the other. If the split extends further posteriorly[1] the new heads become larger than in the last case, but still not full size, Fig. 14.

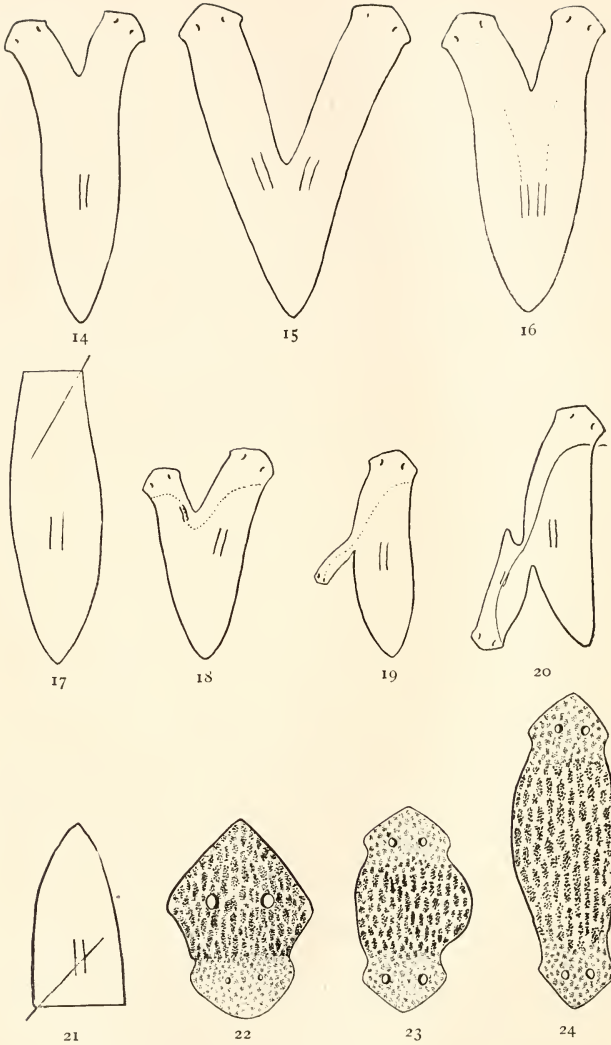
If the cut extends into the region of the pharynx so that two proboscides are formed, one in each half, Fig. 15, the two new heads appear to become larger than in the preceding case, but still do not attain full size. Each appears to be proportionate in size to the part of the body on which it is found, and its size is

[1] The old head had been first cut off.

determined by that of the rest of the part. If the latter should grow to the normal full size the heads would, no doubt, also become full size. Since the parts would become full size if they were separated from each other the explanation of their failure to enlarge beyond a certain size is unquestionably connected with the fact of their union with each other. Very often the pieces partially reunite after the operation, and two proboscides are formed as shown in Fig. 16. In such cases the body at, and beyond, the region of the proboscis is broader than normal, and in consequence the heads at the anterior ends may be larger than when only a single proboscis is present, as in Fig. 14.

In order to see how important a factor the extent of the surface regenerating a head is, as compared with the size of the area of union of the parts, the following experiment was made. The old head was first cut off, and then by an oblique cut, as shown in Fig. 17, a triangular piece was partially cut off. Pieces of this sort tend to unite and must be for a time kept apart. The new head develops on the anterior cut surface of the triangular piece, and the other head on the anterior edge of the oblique cut surface. A new pharynx develops along the posterior edge of the oblique cut in some cases, Fig. 18 and 20, in others not, Fig. 19, depending, in part, on the extent to which the pieces are kept apart after the operation, in part on the nearness of the cut to the region of the old pharynx. As soon as the two new heads have been fully formed it is seen that their size bears no relation to the fact that one has developed on the cross-cut surface and the other on the oblique surface. Their sizes depend rather on the size of the part from which they have developed, Figs. 18, and 19, and whether a new pharynx has appeared in the triangular piece, and also on the relation of the part to which they belong to the rest of the worm. The following examples may make this clearer. In the first case, Fig. 18, the triangular piece has rather a broad attachment to the other part and contains a pharynx. The larger of the two heads belongs to the old part. In the second case, Fig. 19, the triangular piece is much smaller and ends in a smaller head. It does not contain a pharynx and is attached to the other part by only a narrow area.

The third case, Fig. 20, shows that the triangular piece is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united to the other part by only a narrow connection. It contains a pharynx, and a new tail has begun to grow out near the area of attachment. This piece pulled apart later. Practically the same results were obtained when the pieces were cut as shown in Fig. 21. In this case the worm was first cut in two near the old pharynx and then by means of an oblique cut, the triangular piece was separated in such a way that a part of the old pharynx was left in each piece. A new pharynx developed in both pieces, and the same relations between the sizes of the new heads, that were described in the first and third cases above, hold here also.

Conclusions.

The results show clearly that the presence of a pharynx in a new part is an important element in the subsequent growth of the part. A part containing within itself a new pharynx behaves more like an independent worm. The most natural interpretation would be, perhaps, that this is due to the part being able to feed for itself; but while this may to some extent account for the result, yet is probably not the whole explanation. My reason for thinking so is that when the animal feeds the digestive tract of the side piece is also filled with food material. If, therefore, the planarian is kept well fed, the side piece does not lack food material even where there is no pharynx in this part.

There are some facts in connection with the mode of regeneration of planarians that may throw some light on this lack of enlargement of pieces without a pharynx. If the anterior end of a worm is cut off, the new pharynx appears, in the anterior piece, at the posterior edge of the old material. It is, at first structurally, too, the head. If the piece is fed so that the old part loses as little as possible, a new region is intercalated in front of the pharynx, and this region continues to enlarge until the normal proportions have been attained. Again, if a piece of *Planaria lugubris* is cut off behind the old pharynx, a new head arises at the anterior end, and a new pharynx, also at the anterior end just at the border between the new and the old parts. The head and pharynx are in this case also too near together, but a new region of growth is established between the head and the pharynx so

that the two are carried further and further apart. These results show that the principal growing region in the new worms lies in front of the pharynx, and it is conceivable that in the absence of the pharynx this region of growth is not formed.

The influence of the width of the region of attachment—a factor to which I ascribed some influence in my last paper—should also be taken into account here. If a pharynx is present in the part the area of union seems to play a less important rôle than when there is no pharynx present. It is probable that it is not simply the area of union as such that plays the important part, but it is the connection between the internal organs—possibly the digestive tract—that is chiefly involved in the result.

The Formation of Heteromorphic Heads in Planarians.

The formation of a heteromorphic head in *Planaria lugubris*, when the old head is cut off just behind the eyes, has been described in several of my preceding papers. In the past summer I succeeded in obtaining one such case in *Planaria maculata*, but only after a large number of trials, and furthermore in this successful case the cut was not immediately behind the eyes, as seen in Fig. 22. At the same time I cut a large number of worms into short cross-pieces, keeping all pieces of the same length together. To my surprise I found that the only pieces that produced a head at the posterior end, as well as at the anterior end (Figs. 23 and 24) were those taken just behind the old pharynx in the region of the reproductive pore. Two possibilities suggest themselves, viz., the presence of the reproductive organs in the piece, or the cut being made through the region at which this planarian pinches off pieces of itself to form new worms. If the posterior edge of the cross-piece lay in the region of constriction, where the new head of the new worm would develop, it is conceivable that this might cause the development of the heteromorphic head in the cross-piece. A similar series of cross-cuts were made on *Planaria lugubris*, but double-headed pieces were never formed on the cross-pieces from the region of the reproductive pore. In this species, however, new worms are not formed normally by pieces constricting off from the posterior end.

Neither of these possibilities seems to me to give a satisfactory

explanation of the presence of a heteromorphic head. In order to see if any internal condition is present in these double forms that may account for the results, the two preceding pieces from the posterior end, as well as two other similar pieces, were cut into sections. In three of these no pharynx was present, but parts of the old reproductive organs were present in the middle of the piece; in the fourth a pharynx was present, but the pharyngeal chamber did not open to the exterior. There was nothing in the presence of the digestive tract to give a clue as to the cause of the development of a heteromorphic head. It is true that the branches of the digestive tract were united behind, but this in itself furnishes no proof that such a union can account for the result. The sections show that a brain, nerve-cords, and eyes are present in both heads. Whatever the factors are that determine the result, the fact that the heteromorphic head appears only in short pieces will probably also have to be taken into account, for it is very improbable that a heteromorphic head would appear if whole worms were cut through in this region.

Bryn Mawr, Pa., May 25, 1902.

PRELIMINARY NOTE ON THE PRESENCE OF A NEW GROUP OF NEURONES IN THE DORSAL ROOTS OF THE SPINAL NERVES OF THE WHITE RAT.

SHINKISHI HATAI.

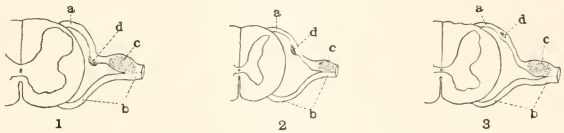
(From the Neurological Laboratory of the University of Chicago.)

While the writer was examining a large number of preparations of the spinal cord of the white rat at different ages, in order to determine whether the so-called "Hoffmann's nucleus," described recently by von Kölliker ("Weitere Beobachtungen ueber die Hoffmannschen Kerne am Mark der Vögel;" Anat. Anz., Bd. XXI., No. 3 u. 4, 1902), appears in the central nervous system of mammals, his attention was drawn to a group of nerve cells in the dorsal roots of the spinal nerves.

The spinal cord of the white rat examined does not have an isolated group of efferent neurones in the region where it was found in the spinal cord of birds. Hence the nucleus of Hoffmann is not present in this animal. A new group of cells was, however, noticed in the dorsal roots, separated from the spinal ganglia and lying between the spinal ganglion and the cord. The shape and structure of the cell-bodies found in this new group coincides with that of the cells in the spinal ganglia, and further, the size of the cells found in this group is approximately similar to that of the cells in the corresponding ganglion. The cell-body is well formed and contains one large nucleus in which one nucleolus generally, or sometimes more than one, is visible. The stainable masses are present in the cytoplasm, showing a characteristic arrangement; the latter substance stains red with eosin with the same intensity as that of the spinal ganglion cells. In every respect the cells which belong to the new group are similar in appearance to the cells of the spinal ganglion. It is easy to see that the cell bodies are surrounded by a connective tissue capsule. In some instances, the capsule is more highly developed than that of the spinal ganglion cells.

The group just mentioned has been thus far found only in the dorsal roots of young white rats having a body-weight of 4, 5, 6 and 7.5 grams respectively, and in an adult gray rat; suitable preparations from the spinal cord of the adult white rat not being at the moment available.

The exact location of the new group of cells differs at different levels of the spinal cord. The above statements on the general character of the cells found, as well as the following description, is based on a study of the young white rats alone.



Semidiagrammatic illustration of the cross section of the spinal cord of the white rat: 1, lumbar cord; 2, thoracic cord; 3, cervical cord. *a* = posterior root; *b* = anterior root; *c* = spinal ganglion; *d* = new group of the cells.

In the lumbar region, these cells lie in the dorsal root fibers rather near the spinal ganglion, where the root enlarges slightly and forms an extension ventrally (Fig. 1). This local enlargement, however, is due to the fact that at this point the dorsal root fibers take a somewhat wedge-shaped course and the nerve cells lie around the pointed tip of the wedge. The total number of the cells seems to vary according to the individual. A single section, 6 micra thick, contains usually one or two cells, though sometimes more can be seen. Since the preparations employed were not made for the purpose of the present investigation and did not form a complete series, the writer was unable to determine the total number of the cells contained in one nerve root. In one case 26 cells were counted in twenty-three successive sections through a single root.

In the thoracic region the cells are located in an enlarged portion of the dorsal root as in the lumbar region (Fig. 2). The total number of the cells in the thoracic root seems greater than in the lumbar root.

In the cervical region this cell group is slightly modified. The cell-bodies lie within the dorsal root where it exhibits a slight

enlargement (Fig. 3). In general the cell group is found nearer the cord. In this region, the group seems to contain a smaller number of the cell-bodies than at the lower levels.

From the above it is clear: First, that these cells are located within a definite region in the dorsal root, not scattered, but forming a group. Second, the cells are contained within well-developed capsules as in the case of the mature spinal ganglion cells. The latter fact shows that the cells found in this group are not at this time migrating, since migration could not occur after the formation of the capsule. The detailed investigation of this cell-group is now in progress, and the suggestion of a name for it is reserved until a more complete examination has been made.

Vol. III. *August, 1902.* *No. 4.*

BIOLOGICAL BULLETIN.

ABNORMALITIES IN THE CESTODE MONIEZIA EXPANSA. III.

(*Continued.*)

3. Origin of the Abnormalities.

The preceding description has rendered it evident that the process of proglottid-formation begins deep within the body and only gradually becomes visible on the surface. The external features of the proglottids, their contours and the furrows which appear between them are only indications of the localized growth that has taken place within the body. Since the primary region of proglottid-formation is the central parenchyma, and the process extends only secondarily to the peripheral parenchyma, it is theoretically possible that a proglottid might be formed in the central parenchyma without becoming visible on the surface as an area of localized growth bounded by furrows. There is some evidence that such a condition occasionally exists. Its discussion is postponed to a later section of this paper. In general, however, the furrows and their external features are simply the visible signs of internal processes of certain kinds and intensities. This being the case, variations in form and structure of the proglottid are the consequence of variation in the formative processes.

(*a*) *Variation in Form of Proglottids.*

The division of the variations into two groups, non-spiral and spiral variations, which was adopted in Parts I. and II., is retained here for convenience. The imperfect or partial proglottids are considered first as showing the simpler forms of variation. A study of the abnormalities of this group shows very clearly that all are simply incompletely developed proglottids, either fused with others or separated from them by furrows.

Part I. (Child, '00) was devoted to the description of cases of this kind and the Figs. 2–23 show a large variety. Bearing in mind the method of formation of each proglottid from the two distinct lateral groups of nuclei in the central parenchyma, *i. e.*, from two separate right and left halves which later become united, it is not difficult to understand how the conditions represented in Figs. 2–23 have arisen.

The cases which differ least from the normal form are those in which the furrows between two proglottids are interrupted at some point or points (*e. g.*, Figs. 11, 12, 13, 15, 16, etc., Part I.). In these cases the proglottids arose in nearly the normal manner. The growth of the two, however, has not been sufficiently distinctly localized to cause the appearance of complete furrows. The various degrees of fusion are indicated in each case by the extent and distinctness of the furrows and the condition of the genital organs. The furrows on the two surfaces of the body may or may not correspond in cases of this kind, *i. e.*, the growth may be more distinctly localized on one surface than on the other or may be alike on both.

In all of the cases just noted, right and left halves of each proglottid are present, and each must have been represented originally by a more or less distinct group of nuclei. In other cases only the right or left half or smaller fractional portion of the proglottid appears, the remainder not being represented. Such variations are clearly the consequence of the formation and growth of a proglottidal anlage on one side of the neck-region only, no corresponding group appearing on the opposite side (Fig. 18, Pt. I.), or else there are two small groups on one side corresponding to one larger group on the other (Fig. 9, Pt. I.). Whether the partial proglottid is entirely distinct (Fig. 18, Pt. I.) or united with a whole proglottid (Fig. 9, Pt. I.), depends probably upon the time of its formation. If two anlagen are found on one side of the body at the same time that one appears on the opposite side, the two of the one side are likely to become connected with the one of the other, thus producing what appears to be a partial division of the proglottid in the transverse plane (*e. g.*, Figs. 9, 10, etc., Pt. I.). If, however, an anlage appears on one side without any corresponding to it on the opposite side, a partial pro-

glottid wholly separated from all others by furrows will probably be formed (*e. g.*, Fig. 18, Pt. I.). These variations consisting of half-proglottids or smaller fractional portions cannot be sharply separated from the preceding group in which both halves are present, but imperfectly defined. As is evident from the figures, all gradations occur. The one extreme approaching the normal condition consists of two or more well-defined proglottids with scarcely interrupted furrows (*e. g.*, Fig. 12, Pt. I.). In many other cases both halves of each proglottid are present, as indicated by the genital organs, but those of one side, right or left, are not sufficiently distinct to cause the appearance of normal furrows (*e. g.*, Figs. 11 and 16, Pt. I.). In most of these cases one of the imperfectly separated halves is reduced, the reduced portion being always the anterior, *i. e.*, the later in time of formation (Figs. 10 *b*, 16, 17, 19, 21, 22 *c* and *d*, Pt. I.). The opposite extreme is reached in those cases where the proglottid appears only on one side of the body, not being represented in any way on the opposite side (Figs. 9 and 18, etc., Pt. I.).

It should be noted that all imperfect or partial proglottids occur either in the right or left half of the body or in both, never in the middle region only. This fact must be interpreted as indicating that they are the result of imperfect development either of the right or left proglottidal anlage or of both. If the normal proglottid were formed by simultaneous differentiation and growth all the way across the body, there seems to be no good reason why abnormalities should not appear near the median line without reaching the lateral margins as often as they occur in the right or left side. Each proglottid arises, however, from two distinct anlagen placed laterally in the central parenchyma and these unite secondarily at the median plane. Therefore the conditions near the median plane must depend upon the development and growth in lateral regions. On the other hand it will be observed that none of the variations are confined to the extreme margin of the body. All include the region which was occupied by the group of nuclei at the time of proglottid-formation. In the figures this region lies slightly lateral to the ovaries.

It is evident that all the facts concerning position, form, extent, and distinctness of the imperfect and partial proglottids harmonize

with and confirm the observations discussed above, on the development of the normal proglottid.

The examination of imperfect or partial proglottids in the light of the facts gained from the study of normal proglottid-development leads to the following conclusions; the right and left halves of the proglottid pass through at least the early stages of their development independently; either or both may remain incompletely developed, as regards size, form, or distinctness, or only certain parts of one or both may develop, or finally one may be wholly absent. All imperfect and impartial proglottids are the result of such incomplete development, and in their manifold forms illustrate a great number of different combinations of the various factors concerned.

A very early stage of abnormal proglottid-formation is illustrated in Fig. 57. The dotted lines drawn through the figure indicate approximately the boundaries of an abnormal region. On the left side two half-proglottids are forming, and corresponding to these on the right is only one. In this case the growth is almost wholly confined to the central parenchyma, so that furrows on the surface are not yet present. Yet I think that there can be no doubt that this region represents an early stage of a condition similar to that shown in Fig. 9, for example. On the other hand, the account of proglottid development has rendered it evident that the zones of nuclei represented in Fig. 57 develop from the lateral groups of early stages. This case forms in some respects a connecting link between the observations upon normal proglottid-development and the facts gained from the study of older, well-developed abnormalities. It is the only case of abnormal form observed at so early a stage.

The spiral modifications of form were described in Part II. (Child, '00) and include Figs. 24–37. The simplest form of spiral is such as appears in Figs. 24, 25 *b* and 26 (Fig. 26 is reproduced as Fig. 72), viz., a single half or fractional proglottid united on one surface with the proglottid posterior to it, or separated by a furrow as in Fig. 24, and on the other surface with the proglottid anterior to it.

It is of course evident that such a short spiral differs only slightly from a fractional proglottid of the sort which is shown in

Fig. 9 (Pt. I.) for instance, the only difference being that in case of a spiral the fractional proglottid unites with different proglottids on the two sides of the body. It appears probable that in such cases a slight difference in time of formation of the dorsal and ventral portions of the proglottid is the cause of the spiral condition. As was pointed out above, the relation of partial

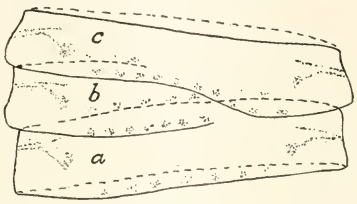


Fig. 72.

proglottids to the proglottids posterior or anterior to them probably depends largely, if not wholly on the time of their formation. If a partial proglottid is formed at the same time as the next posterior proglottid, *i. e.*, a little before normal time, the two will probably be incompletely separated. If the formation of the partial proglottid is retarded slightly it will probably become united with the proglottid anterior to it. And lastly, if the time of formation of the partial proglottid does not correspond to that of any other it will probably be distinct from all others. Applying the same conclusions to the spiral partial proglottids (*e. g.*, Fig. 72) it appears probable that one side of the partial proglottid the dorsal (upper) side of *b* in the figure is formed at about the same time as the proglottid posterior to it, whereas the ventral (lower) side corresponds in time of formation with *c* and so becomes united with it. Recalling the method of formation of the proglottid, it is easy to see that such differences in time of development of different parts are at least theoretically possible. The localization of growth, which is the essential feature of proglottid-formation, may for some unknown reason appear somewhat earlier on one side than on the other, just as it appears earlier in the lateral than in the median regions of the central parenchyma.

If now the dorsal and ventral sides of a series of anlagen on either the right or left side of the body show similar differences

in time of development, a series of spirals may be formed such as appears in Fig. 35 (Pt. II.). If the development of the dorsal and ventral portions of both right and left anlagen is accelerated or retarded the parts show normal relations to adjacent proglottids.

In some cases a form of spiral somewhat different from that just described is produced; in this the furrows on one surface of the body bend either anteriorly or posteriorly near the right or left margin and so unite with other than the furrows corresponding in position on the opposite surface.

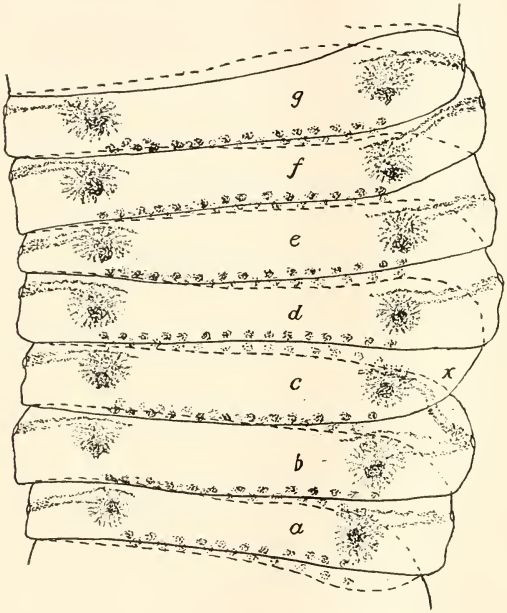


FIG. 73.

The only well-developed case of this kind was shown in Fig. 36 (Part II.). Another case approaching this condition was shown in Fig. 37. These two figures are reproduced here as Figs. 73 and 74. Both are viewed from the dorsal surface. It is evident from the figures that the growth is incomplete in the region

where the furrows bend. In Fig. 73 the curved portions of the furrows near the right margin of the ventral surface are less distinct than the other portions, *i. e.*, growth has been less all along this region of the body. The appearance of the spiral arrangement is connected with the condition of imperfect development. The proglottid *a*, Fig. 73, being the most posterior of the series, *i. e.*, the first formed, is the starting point of the abnormal arrangement. In this proglottid the anterior part of the right ventral region shows a progressive reduction in size with approach to the margin. Now if the same region in each of the following proglottids shows the same imperfect form the anterior boundary

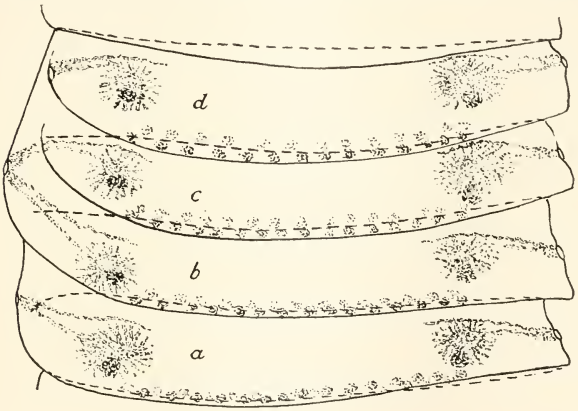


FIG. 74.

will in each case form a curve instead of extending straight to the margin. But the anterior boundary of one proglottid is the posterior boundary of the next anterior; thus the area which would normally be included in *a* becomes the part of *b*, etc. The indistinctness of the curved portion of the furrow is due to the absence of the fold which normally arises at the posterior border of each proglottid. In the regions where the boundary between the proglottids is transverse the fold and inter-proglottidal glands are developed as usual.

In Fig. 74 there is a close approximation to a spiral as the result of incomplete formation of the proglottids. The curve at

the left end of each of the dorsal furrows is clearly due to a progressive reduction and final cessation of proglottidal growth in the region under consideration.

It is the left posterior dorsal portion of each proglottid which has failed to develop fully in this case, while in Fig. 73 it was the right anterior ventral portion. If development in Fig. 74 were complete enough to cause the formation of furrows all the way to the margin a spiral would be formed opposite in direction to that in Fig. 73, simply because of the difference in position and relations of the imperfectly developed regions. But the furrows on the dorsal surface curve to such an extent that they do not reach the margin at all. On the ventral surface there is no reduction of the proglottids, but they are not differentiated at the extreme margin. Consequently the left margin of the region *b-c-d* is not divided into proglottidal regions.

A knowledge of the arrangement of the transverse nerve-commissures in regions of spiral variations of this kind would be most interesting and valuable.

The spiral variations afford further illustrations of the independence of the right and left proglottidal anlagen. In general, the spirals are due to some irregularity in time of growth and differentiation of parts, or to incomplete growth in either the right or left half of the body. A variation of this kind having appeared in a single proglottid shows a certain tendency to reappear on the same side in a number of proglottids (*e. g.*, Figs. 73 and 74).

The condition and development of the furrows cannot be regarded as an exact indication of the conditions in the proglottids which they bound, though a study of the arrangement of the reproductive organs in the abnormal proglottids shows that the furrows do indicate very closely the degree of development or distinctness attained.

But since the earliest stages in proglottid-formation occur only in the central parenchyma, the formation of inter-proglottidal furrows on the surface of the body being a later process, it is evident that a proglottid may attain a certain degree of development without being represented by furrows on the surface. That such cases do actually occur was pointed out in Part I. (*e. g.*, Fig. 10, *b*, p. 226; Fig. 20, *f*, *g*, pp. 240–241). In Figs. 10, *b*, and 20,

f, *g*, there is a partial duplication of the reproductive organs, but no furrow between the two sets. Here the causes leading to localization of growth have not affected the outer parts of the body. The genital organs are always reduced in cases of this kind.

Furthermore, it is possible that the furrow may not coincide perfectly with the boundaries of the proglottid in the central parenchyma. The growth in the peripheral parenchyma may be reduced or modified in such a manner as to change the direction of the furrow so that it no longer indicates the exact boundary. A very distinct case of this kind was shown in Fig. 38, Part II., at *m*, and discussed on page 280, and it is possible that the oblique furrow between *c* and *d* in Fig. 38 is another case (p. 283, Part II.).

It is probable that in many cases furrows which curve anteriorly or posteriorly do not correspond to the exact boundaries of the proglottids. Curved furrows of this kind always indicate incomplete development in the peripheral parenchyma, but the central regions may often be more perfectly developed than the furrows indicate.

The preceding discussion of a few typical cases of form-variation in the proglottids renders it sufficiently evident that all of these variations are due to incomplete development of some part or parts, or of the whole proglottid. One of the two anlagen may be absent or may become united with the next anterior or posterior to it. Either or both may fail to attain full size or normal form.

The inter-proglottidal furrows are the chief external indications of internal conditions of growth and development. But the structure of the genital organs in some of the imperfect proglottids indicates a somewhat more nearly perfect condition than that represented by the furrows.

Throughout the whole series of form-variations no new methods of growth or proglottid-formation are indicated. All variations absorbed are simply failures to attain full development. They illustrate very clearly the independent development of each of the two anlagen.

Variations in the form of the proglottids—abnormalities—are extremely common in *Moniezia*, much more so than in most

Cestodes. This condition is perhaps connected in some manner with the fundamental structural or physiological characteristics of the form.

An inquiry as to the causes of the frequency of form-variations in *Moniezia* may therefore concern itself first with the normal anatomy and physiology. A few suggestions as to some of the possible factors concerned are made here in the hope of throwing some light upon this problem. First, the method of formation of the proglottid, its appearance in two separate parts which later unite, must favor to some extent the development of abnormalities, for a slight variation in position or time of appearance of the anlagen may apparently effect the extension and union of the anlagen of the two sides. It is not known at present whether the proglottids of all other Cestodes arise in the same manner. If in some the proglottids originate in a single group of cells the chances for abnormal development would apparently be fewer. Second, the form of the proglottids, the great width in proportion to the length favors the appearance of abnormalities. In the early stages the proglottids are extremely short and it is evident that abnormal union of right and left anlagen must occur more frequently than in forms with longer proglottids. Third, it is possible that the proglottids of *Moniezia* possess a lower degree of individuality than those of some other Cestodes, *i. e.*, that they are less distinct structurally and physiologically. If this is the case then incomplete separation may be expected to occur more commonly than in forms with more highly individualized proglottids.

The frequency of form-variations differs greatly in different specimens. More than half of the cases shown in Parts I. and II. were taken from a single chain and represent only a part of the abnormalities present. Other specimens possess in most cases only a few abnormal proglottids, but nearly every specimen exhibits one or two. We have at present no clue to the cause of this difference. It is possible that age may have some influence, the older specimens showing a tendency to incomplete development. Injuries to the neck-region, or the repeated tearing away of the whole body, or perhaps malnutrition, may also favor the appearance of abnormalities, and finally these differences

may be due not to age or external conditions but to an individual peculiarity. Some may feel inclined to regard this as a reversion to an ancestral condition in which the division of the body into regions of localized growth was less distinct and regular.

(*b*) *Abnormalities in the Structure of Proglottids.*

Very closely correlated with the variations in form of the proglottids are variations in the structure and arrangement of the reproductive organs. This close correlation was pointed out in detail in all those cases cited in Parts I. and II., in which the reproductive organs were developed. Some points of interest remain to be considered.

The relative position in the proglottid of the various parts of the reproductive organs was noted in Part I. Each portion has a more or less definite relation to the others and to the proglottid as a whole. We might expect that a change in the form of the proglottid would be accompanied either by a modification in the form or arrangement of some part or parts of the reproductive organs, or else, in cases of more extreme variation, that some portions or all of the reproductive organs would be absent. As a matter of fact both of these possibilities are represented.

Considering first the modifications in form and arrangement of the organs without loss of essential parts, we find that considerable departures from the form may occur without apparently interfering with the function of the organs. Two sets of organs may open through a single pore (Figs. 11, 22, *a*, *b*, Pt. I.; Figs. 36, *b*, *c*, 37, *b*, *c*, Pt. II.). In such cases the direction of the ducts in at least one of the sets of organs is distinctly different from the normal direction, being more oblique. In other cases the ducts may run very obliquely toward the margin but without uniting with others (Fig. 33, *c'*, *d'*; Fig. 39, *a'*, Pt. II.). In cases of this kind the ducts may be and usually are much longer than the normal ducts. In other cases the organs may be nearer the margin than normally and the ducts are then shorter than normal. The vas deferens may open directly into the seminal receptacle as in Fig. 21, *c'* (Pt. I.), or two vasa deferentia or two oviducts may unite (Figs. 21, *a'*, 40, 41).

Reduction in size of the female organs and reduction in the

number of the testes frequently occurs in proglottids of less than normal size.

This brief review of these cases only serves to call attention once more to the fact that was repeatedly pointed out in Parts I., and II., viz., that the form and arrangement of the reproductive organs may vary widely in correlation with variations in form of proglottid.

In case the form-variation is extreme, however, parts of the reproductive organs may remain undeveloped and these parts may be those which are essential for the normal functioning of the system. The consideration of these cases leads to the very important conclusion that the development of each part of the reproductive organs shows a very close correlation with the development of the particular region of the proglottid in which it lies. This was pointed out repeatedly in Parts I. and II., and it is not necessary to go over all the particular cases again.

The different conditions represented are manifold. Apparently any portion of the organs may fail to appear as this or that portion of the proglottid fails to develop fully. In case the dorsal side of the proglottid is distinct and the ventral is not, only the vas deferens and testes appear, these lying in the dorsal region of the proglottid (Figs. 16, *b'*; 17, *b'*, etc., Pt. I.). If the ventral side only is distinct from the adjacent proglottids only the female organs or parts of them appear (*e. g.*, Fig. 22, *d*, Pt. I.). Ovary, testes, and, in some cases, the inner portions of the vas deferens and vagina[1] may be present, but if the marginal region is dwarfed the middle, or terminal portions, or both, of the ducts may be entirely absent, or present only in part (*e. g.*, Fig. 39, *b*, Pt. II.). And finally, in extreme cases the reproductive organs may be represented only by one or two small groups of cells in the region where the ovary or vitellarium would appear if present (Fig. 18, Pt. I.).

It is rather common in cases of this kind to find a marginal pore and perhaps the cirrus present but entirely unconnected with the inner portions of the reproductive organs. Either the ducts

[1] In Parts I. and II. the designation "oviduct" was incorrectly applied to the whole female duct, including the vagina. Of course the oviduct proper is only a small portion of this. I do not think that my use of the word was such as to cause confusion in this particular case, but I desire to call attention to the error.

leading to the pore are undeveloped (Figs. 22, *d*, Pt. I.; 39, *b*, Pt. II., etc.), or else they open through another pore (Fig. 21, *a'*, Pt. I.; Fig. 40, *b*, Pt. II., etc.). There is in these cases no indication of any morphological connection between the pore and any other parts that may be present, but it may be that the presence of the internal reproductive organs favors in some manner the formation of a pore (though not essential). While this condition occurs so commonly, its complement, development of the middle portions of the ducts without a pore at their outer end or the internal organs at their inner end, has never been observed by me. Thus there is apparently a certain tendency for the organs in the central parenchyma and the marginal pore to appear, even without connection, while the middle portions of the ducts never develop alone. In other words the central parenchyma and the margin are regions of "least resistance." I think the absence of the middle portions of the ducts in so many cases may be due in part to the fact that these portions traverse the thick muscle layers which lie between the central and peripheral parenchyma. The ducts, like the other portions of the organs, appear first as aggregations of parenchyma-nuclei in a mass of undifferentiated cytoplasm. Very few or none of these nuclei exist in the muscle layers. Those portions of the ducts which penetrate the muscles appear normally somewhat later than the outer and inner parts, apparently because the material is less abundant here and probably also because the muscle-layers offer a certain degree of resistance to the penetration of the ducts. Apparently either the outer or inner portions or both grow into the muscle-layer and so become continuous. Now it is extremely probable that if any tendency to incomplete development of the reproductive organs exists in the lateral regions it will show itself first in the region where material is least abundant and growth apparently most difficult, *i. e.*, in that portion of the ducts which lies in the muscle-layers. This portion might, therefore, fail to appear, whereas the outer and inner parts, lying in regions where material is more abundant and where no special resistance to growth exists, probably do not require so strong a stimulus for their development.

The various abnormalities in the reproductive organs afford

very strong evidence in favor of the view that the principal parts of the system develop independently. A glance over the figures will show that almost any portion of the organs may be present alone. As noted above, the middle portions of the ducts never appear alone and it may be that the ovary and vitellarium always appear together. The dependence of the middle regions of the ducts on other portions has been discussed. The relation of ovary and vitellarium is perhaps explicable on the view commonly held that the vitellarium represents a modified portion of the ovary. The earliest stage of the reproductive organs is a mass of nuclei in the region where the ovary and vas deferens appear later. This increases in size and gradually differentiates, but the study of the abnormalities renders it very evident that the development of the portion which forms the vas deferens does not depend upon the development of the ovarian portion and *vice versa*, for the one portion may appear without the other, although both appear to differentiate from a common mass. Testes and vas deferens develop independently of each other, for testes appear in proglottids where there are no vasa deferentia (Fig. 39, *b*; Pt. II.) and *vice versa*. And finally, the pore and terminal portions of the ducts develop independently of the other organs. In the normal proglottids the group of nuclei representing the pore, cirrus, etc., is entirely unconnected in early stages with the group representing the inner organs and may appear alone (Fig. 19, *b*; Pt. I.). The connecting portions of the ducts appear later, as was mentioned above.

We must conclude, therefore, that the lateral reproductive organs are formed from at least three distinct "centers" or regions of independent growth. These may be called the vas deferens region, the ovarian region, and the terminal region. There may be others besides these. In addition, the testes develop in the central parenchyma independently of each other and of the lateral organs. All cases of the incomplete development of the reproductive organs are to be regarded as due to the incomplete development or total obliteration of one or more of these regions.

It is important to note that in all cases of partial development the portions of the reproductive system present show approximately the same degree of differentiation as the corresponding

parts in adjacent normal proglottids, so that it is possible to determine in the older proglottids just what parts are represented in each case. Evidently then there is no great amount of retardation in development in the partial or otherwise abnormal proglottid. Organs once formed develop with the same rapidity as those under normal conditions. What is lacking in these abnormal proglottids is the initial stimulus or the proper condition for the *formation* of this or that portion. The structure of each abnormal proglottid is as complete as possible for that particular form of proglottid. Organs are formed at the same time as in other proglottids and those organs which do not appear at the normal time are not formed later. There is no decrease in the rapidity of development, there is simply the presence or absence of a certain form and correspondingly the presence or absence of a certain structure.

In Figs. 37–41 (Pt. II.) variations of another sort in the reproductive organs are shown. Figs. 37 and 38 both show cases of transverse duplication of portions of the reproductive organs on one side of the body. This abnormal condition does not find a satisfactory explanation in the form of the proglottid. All that can be said at present concerning it is that for some reason an extra growth-center, or perhaps more than one, is formed. In the two cases cited this gives rise only to female organs, in one case (Fig. 38, *b*) to a small imperfect ovary and a few groups of cells representing the vitellarium, in the other (Fig. 39, *c*) to these organs, and, in addition, a small blind seminal receptacle. I have been unable to discover any clue to the causes concerned in the formation of these supernumerary organs.

In Figs. 40 and 41 (Pt. II.) three cases of reversal in position of the lateral organs appear. In Fig. 40 the organs of the right side are reversed in proglottid *b*, and in *d* those of the left side. In Fig. 41 another case of the same kind occurs. This peculiar variation is evidently correlated with the abnormal form of the proglottids. It is, however, difficult to understand why a reversal should occur in these cases, and not in others where conditions are closely similar. It does not seem probable that these variations indicate a "reversal of polarity" in those parts of the proglottids containing them. Considerable variation in the posi-

tion of the organs occurs in normal proglottids. These appear to be extreme cases of adaptation in growth to abnormal conditions.

The inter-proglottidal glands show numerous departures from the typical arrangement and these variations are very closely correlated with the form-variation of the proglottids. The inter-proglottidal glands lie along the posterior border of each proglottid just anterior to the furrow and open into the deepest part of the latter. They are absent near the margins of the body. In general the glands appear wherever even a small portion of a furrow extends beyond the marginal region in which they are never found. The glands never appear except in connection with a furrow, and they appear only along furrows which are transverse or nearly so. In one case shown in Fig. 38 (Pt. II.) the furrow becomes oblique, but the glands tend to continue in a transverse row, and at the anterior end of the oblique portion lie at some distance from the furrow and are connected with it by long ducts, while at the posterior end one gland lies posterior to the furrow instead of in its normal position anterior to it. With respect to this case the suggestion was made on page 283, Part II., that the rows of glands may coincide with the proglottidal boundaries while the oblique portion of the furrow does not. The glands tend to form along the boundaries but apparently do not form at all unless a furrow is present.

The numerous variations which have formed the subject of this paper have demonstrated very clearly the close relation between form and structure. It remains for us to consider whether the variations in structure are the result of the form-variations or whether some common cause underlies both.

The organs of the proglottid appear considerably later than the proglottid itself. If variations in their form and arrangement are due to the same causes as the variations in form of the proglottid, these causes must be active after the formation of the proglottid and must affect the development of the organ in such a way as to cause an apparent adaptation to the form of proglottid.

The natural and obvious conclusion is, I think, that the development of the proglottidal organs must depend upon the devel-

opment of those regions of the proglottid in which they lie. Reduction of any given region means reduction in amount of material and in space, and the organs which develop in this region must be affected. The organs of the normal proglottid are the only organs possible under the given conditions of form, size, etc. Change in the conditions must produce alterations in the organs. The correlation between form and structure as shown in the variations is so close that, given a proglottid of a certain form, it would be possible to predict with a considerable degree of accuracy in most cases what the condition and general arrangement of the organs would be. It is scarcely necessary to seek for a common cause for this correlation of form and structure. I think it is sufficiently clear that the variations in form of the proglottids are the cause of the variations in structure and arrangement of the proglottidal organs.

HULL ZOOLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO, March, 1901.

LITERATURE.

In the references to literature I have followed the plan proposed recently by Bather ('01), viz., the use of the last two numerals denoting the year for all papers which have appeared within the hundred years preceding the paper.

Bather, F. A.
'00 A Bibliographic Catch Title for Ever and Ever. Science, N. S., Vol. XIII., No. 319.

Child, C. M.
'00 Abnormalities in the Cestode Moniezia expansa. Biol. Bull., Vol. I., Nos. 5 and 6.

Cohn, L.
'98 Untersuchungen über das Centrale Nervensystem der Cestoden. Zoöl. Jahrb.: Abth. f. Anat. and Ont., Bd. XII., H. I.

Koehler, E.
'94 Der Klappenapparat in den Exkretionsgefassen der Tänien. Zeitschr. f. wiss. Zoöl., Bd. LVII.

Lühe, M.
'96 Das Nervensystem der Ligula in seinen Beziehungen zur Musculaten. Zoöl. Anz., Bd. XIX.

Scheibel.

'95 Der Bau der Tænia Magna Abilgaard. Inaug. Diss. Giessen.

Tower, W. L.

'00 The Nervous System in the Cestode Moniezia expansa. Zoöl. Jahrb.: Abth. f. Anat. and Ont., Bd. XIII., H. 3.

Descriptions of Figures.

Fig. 42. Transverse section through the neck near scolex.

Fig. 43. Transverse section from middle of neck region.

Fig. 44. Frontal section through anterior part of neck.

Fig. 45. Sagittal section near median line from near middle of neck region.

Fig. 46. Diagram indicating the levels from which various figures were taken. The numbers correspond with the numbers of the figures.

Fig. 47. Neck, in toto, near scolex.

Fig. 48. Neck, in toto, near middle.

Fig. 49. Neck, in toto; formation of proglottids beginning.

Fig. 50. Neck, in toto; proglottids distinct in central parenchyma.

Fig. 51. Neck, in toto; proglottid-formation extending to peripheral parenchyma.

Fig. 52. Neck, in toto; inter-proglottidal furrows distinct.

Fig. 53. Diagrammatic transverse section through neck, indicating the planes of the sections shown in various figures. The numbers correspond with the numbers of the figures.

Fig. 54. Diagram of a portion of the neck-region, indicating the planes of certain sections.

Fig. 55. Section (nearly sagittal) through one side of posterior part of neck.

Fig. 56. Section (nearly sagittal) through one side of posterior part of neck.

Fig. 57. Frontal section, showing early stages in proglottid-formation. The dotted lines enclose an abnormality.

Fig. 58. Sagittal section of a stage in which the inter-proglottidal furrows first become visible.

Fig. 59. Frontal section, showing one side of young proglottids.

Figs. 60–70. Surface-views and sagittal sections of proglottids of various ages, showing the relative increase in size of different regions.

Fig. 71. Sagittal section, showing an early stage in the formation or the posterior proglottidal folds.

Fig. 72. Reproduction of Fig. 26; a simple form of spiral abnormality.

Fig. 73. Reproduction of Fig. 36; a spiral series.

Fig. 74. Reproduction of Fig. 37. Incomplete development approaching spiral form.

THE TOPOGRAPHY OF ORGANS IN TYPICAL SEGMENTS OF HIRUDO.

BENNET M. ALLEN.

I wish, first of all, to acknowledge my indebtedness to Prof. C. O. Whitman for the incentive to this work, and for much kindly assistance. The work was begun in the hope that it might throw some light upon the question of the limits of the somite in the leeches. No attempt will be made to discuss this question. I shall merely endeavor to describe the position of organs in relation to the annuli, in the hope that this may be of some assistance to future workers in the field of metamerism.

The 15th, 16th, and 17th segments were taken as typical in this species (*Hirudo medicinalis*). These segments are in that region of the body where the original position of parts has been least modified by displacement, fusion or otherwise.

Gross dissections were first carefully studied, and then the more exact location of organs was determined from a study of thick sections, both longitudinal and sagittal. The leeches were killed in about 0.05 of one per cent. solution of chromic acid, in which they died in an expanded condition. Immediately after opening them by a dorsal median cut through the body-wall, the latter was pinned back on each side, or else two lateral cuts were made and the entire dorsal portion of the body-wall removed. In the latter case one could better observe the relation of the organs to the sensory ring which had been previously notched at each side. In preparations intended to show merely the alimentary canal, the blood, which is usually found to distend it, served, when hardened by the reagents used, to keep the canal distended and thus to preserve its shape.

In case organs lying below the alimentary canal, such as nerve cord, testes, etc., were to be studied, the canal was at once opened dorsally and the blood washed out. The preparation was then kept for a few hours in water, and the organs to be studied were exposed by the removal of connective tissue. Extreme care was necessary in order that the organs should not

be displaced by this work of dissection. Preparations were preserved in formalin or alcohol.

The material to be sectioned was hardened in 3 per cent. chromic acid for a day or two, and after the usual washing and dehydrating, cleared in xylol and embedded in *soft* paraffine. Very thick sections were cut, cleared in xylol and mounted serially in Canada balsam. They were studied under a dissecting microscope with fairly high power lens. The relation of particular organs to the limits of the rings was carefully estimated by placing under the slide a piece of thin paper with straight ink-marks across it.

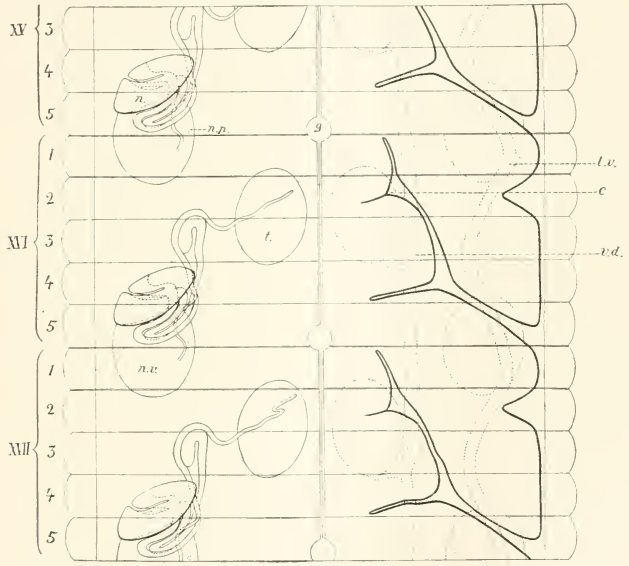
There was considerable variation in the position of the nerve ganglion with reference to the sensory ring of the somite to which it belonged. From a table of measurements I selected the specimen which showed an average position of the ganglia. The three ganglia in this specimen (K) all projected beyond the anterior limit of the sensory ring to which they belonged, and lay largely in the fifth ring of the preceding somite. The proportion thus found in front of the limit of the somite was 0.7 in the case of the ganglion of the sixteenth; 0.8 for that of the seventeenth; and likewise 0.8 for the ganglion of the eighteenth somite. The observations extended over a series of thirteen specimens. The measurements, similarly taken in all specimens, were as follows:

Somite.	16th.	17th.	18th.
A	1.0	0.6	x
B	1.0	0.8	1.0
C	1.0	1.0	1.0
D	1.4	1.3	1.0
E	0.6	0.7	x
F	0.6	0.6	0.8
G	0.7	1.8	1.6
H	0.5	0.5	0.8
I	0.7	0.8	0.5
J	0.0	1.2	1.7
K	0.7	0.8	0.8
L	0.5	x	0.8
M	0.3	0.7	0.9

NOTE.—No. 1.0 indicates that the ganglion in question lies wholly within the fifth ring of the preceding somite; a decimal

fraction shows the proportion of the length of the ganglion lying within it; while a figure like 1.5 shows that the posterior part of the ganglion lies 0.5 of its total length in front of the limit between the sensory ring and the fifth ring of the preceding somite.

By means of the sections the position of the ganglia and the prominent constriction of the alimentary tract (*C*) was in each case determined. For the position of the other organs, I relied upon a study of the gross dissections.



Segments of XV, XVI and XVII of *Hirudo medicinalis* (× 12).

The outline of the alimentary tract is shown by the heavy line on the right side of the plate. Structures underlying other organs are shown by broken lines. Organs or parts of organs lying beneath portions shown by broken lines, are represented by dotted lines.

n, nephridium; *np*, nephropore; *t*, testis; *g*, ganglion; *c*, constriction of alimentary tract; *nv*, nephridial vesicle; *vd*, vas deferens; *lv*, lateral vessel.

The results can be best studied by referring to the accompanying plate. In the region presented by it, the alimentary tract consists of a rather narrow tube with two pairs of out-

growths in each somite. Attention may first be called to the place marked (*C*). This is a very pronounced constriction occurring similarly in each of the somites studied. It is far more marked than the other constrictions in the somite. This probably marks the primitive metameric limits of the alimentary tract. This is merely a suggestion, as a study of the embryology of this form would be necessary, before a definite statement could be made in regard to this point. The more prominent of the two sets of pouches are those opening into the alimentary tract at the posterior end of each somite. The testes (*t*) are situated chiefly in the second and third rings, their ends projecting slightly into the adjoining rings. The vas deferens connecting them is shown only on the right-hand side of the drawing.

The nephridia, shown on the left-hand side of the drawing, are much coiled, extending from the nephrostome at the tip of the testis lobe in the front part of the second ring, to the end of the duct by which the nephridial vesicle opens to the exterior through the nephropore. This nephridial vesicle lies in the fifth ring of the somite to which it belongs and the sensory, or first ring of the somite immediately posterior. It projects slightly beyond each of the two rings named. The nephropore is situated in the extreme posterior part of the fifth ring.

Hull Zoölogical Laboratory,
University of Chicago.

THE ARTIFICIAL PRODUCTION OF SPORES IN MONAS BY A REDUCTION OF THE TEMPERATURE.

ARTHUR WHITE GREELEY (FROM THE HULL PHYSIOLOGICAL LABORATORY OF THE UNIVERSITY OF CHICAGO).

The processes of reproduction among the Protozoa present many interesting problems from the physiological point of view. Aside from the so-called sexual reproduction, which is always preceded by the conjugation of two similar or slightly differentiated individuals, there exist many forms of asexual reproduction, varying from the simple division of the parent organism into two, or in some cases as many as eight, daughter cells, to the more complicated processes of encystment and spore formation. The processes of encystment and spore formation may be preceded by conjugation, but in most cases they are simply a direct transformation of the motile organism into a resting form. Surprisingly little is known about the physical and chemical conditions which determine the transformation of Protozoa into cysts and spores.

It has generally been observed that when a pond, in which the organisms live, begins to dry out, cysts are formed. This fact caused Rhumbler and Cienkowsky[1] to undertake a series of experiments. They kept cultures of Infusoria in small, loosely covered dishes, and allowed the water to gradually evaporate. Before the evaporation had become complete, all the Infusoria had formed cysts. Doflein[2] has repeated these experiments and has confirmed the results of Rhumbler and Cienkowsky. He found that after a long-continued evaporation of water the cysts would further break up into many small spores, thus proving that the processes of encystment and spore formation are identical. Other investigators attributed this encystment to a lack of oxygen, rather than to the evaporation of the water. Maupas[3]

[1] Cienkowsky, *Archiv. für Mikroscopische Anatomie*, 1866, I., p. 203.

[2] Doflein, *Archiv für Protistenkunde*, 1902, I.

[3] Maupas, *Archives de Zoölogie Experimentale*, 1888, VI., p. 165.

has kept cultures of various Infusoria for long periods of time, and has found that some of the carnivorous forms, notably *Oxytrichia*, form cysts after they have been deprived of food. Hertwig[1] has observed that the same fact holds good for *Actinosphærium*. In the same series of experiments he found, however, that the consumption of an excess of food may cause encystment as well as starvation. Other cases are on record also in which various carnivorous Infusoria have been seen to encyst after engulfing a large amount of food. Klebs,[2] in a large number of experiments on fresh water Algæ, has observed that in *Vaucheria* zoöspores are formed when the filaments are transferred from the light to the dark. Klebs reared *Vaucheria* in the following solution, used at concentrations of from 0.1 to 0.4 per cent.: — $Ca(NO_3)_2$, 4 parts; $MgSO_4$, 1 part; KNO_3, 1 part; K_2HPO_4, 1 part; and found that when the filaments were transferred from this solution to distilled water, irrespective of light or dark, zoöspores were formed. He also succeeded in producing parthenogenetic spores in *Spirogyra* by plasmolyzing the cells with a sugar solution. Klebs makes the general statement that the formation of zoöspores in *Vaucheria* is aided by lowering the temperature, but made no experiments to show that a lowering of the temperature itself will actually cause the plant to form spores. He apparently had in mind only the limits of temperature at which spore formation may take place, when the process is initiated by other means. Beyond these observations, which are rather inconclusive as far as the Protozoa are concerned, nothing is known about the conditions which determine encystment or spore formation.

Dr. Loeb suggested that I take up the problem of asexual reproduction from an experimental point of view. Many authors had noticed that the mode of reproduction changes in certain aquatic animals or Protozoa when the pond in which they live begins to dry out. But the question was, how can the lack of water in a pond interfere with the mode of reproduction? Dr. Loeb's idea was that the real physical factor at work in this case

[1] Hertwig, *Sitzungberichte der Gesellschaft für Morphologie und Physiologie in München*, 1899.

[2] Klebs, *Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*, Jena, 1896.

was the rapid or extensive changes of temperature. As long as the bulk of water in a pond is large, the daily changes of the temperature of the air will only cause a slow or slight variation in the temperature of the pond. But where the bulk of water is small, the temperature of the latter will follow rapid changes in the temperature of the air more rapidly and completely. In order to test this idea, he suggested that I try whether or not, through sudden changes of temperature, organisms might be caused at any time to reproduce asexually instead of sexually. The experiments were first performed on *Stentor* with the results already described in a previous paper.[1] It was found in these experiments that by lowering the temperature the animal would go into a resting stage, which in appearance resembled a cyst; but in no case did I obtain spores. These results could not be obtained by raising the temperature.

During the past year the low temperature experiments have been continued on several other Protozoa, and in all of them structural changes similar to those already described for *Stentor* have been obtained. But in one form, *Monas*, our original purpose has been carried out, namely, the artificial production of spores by means of variations in the temperature.

Monas is a small flagellated Infusorian, of an exceedingly simple structure, and occasionally appears in great numbers in cultures that have been prepared for *Paramæcia*. It can be easily maintained in the laboratory in great quantities by adding to the culture from time to time a little bread, upon which the Monads

FIG. 1. The adult *Monas*.

thrive surprisingly. In all the experiments the Monads were isolated in small covered dishes, and the supply of water kept constant by frequent renewal from the aquaria in which the animals had been reared. The temperature was lowered to the desired point by placing the dishes in a refrigerator in which con-

[1] Greeley, *American Journal of Physiology*, 1901, VI., p. 122.

stant temperatures, ranging from 1° to 10° C., could be maintained. For each low temperature experiment, a control experiment was performed at the temperature of the room, and great care was taken that all the conditions, with the exception of temperature, might be identical in the two cases.

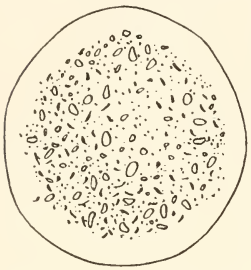


FIG. 2. A resting cell of *Monas*, formed by an exposure to a temperature of 4° C. during six hours.

Monas is more sensitive to changes in the temperature than any of the other Protozoa experimented with. Within a few hours after the temperature has been lowered to 4° C. all the Monads in a dish settle to the bottom, and cease their progressive movement. At same time the cell gradually becomes spherical, the flagellum and mouth opening disappear, and there is formed a resting cell like those already described in the experiments upon *Stentor*. These resting cells can be kept at a temperature of 4° to 6° C. indefinitely, and will withstand partial desiccation without losing their power to revert to the normal *Monas* form when they are removed to the temperature of the room. This reversion to the motile form takes place within twenty-four hours after the room temperature has been reached. The flagellum first makes its appearance, and the cells become motile while still in the spherical conpition. They soon, however, assume the normal elongated form of the adult *Monas*.

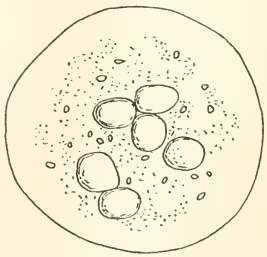


FIG. 3. The formation of spores within the resting cell, after an exposure to a temperature of 1° C. for five days.

If these resting cells of *Monas* which have been formed at a temperature of 4° to 6° C., instead of being returned to the temperature of the room, be placed on ice at a temperature of 1° C. further structural changes take place as a result of this extreme lowering of the temperature. After remaining at a tem-

perature of 1° C. for five to seven days, the protoplasmic contents of the resting cells break up into small spherical spores, from two or three to twenty-five in each cell. In most cases these spores are discharged from the resting cells as soon as they are formed. They have thick cell walls and are non-motile. They may be kept indefinitely at any temperature below 8° C. and withstand desiccation perfectly.

When the spores are removed to the temperature of the room and isolated in small closed cells under the microscope their development into the motile Monad can be easily followed. The first attempts to demonstrate the development of the spores failed in several instances because of a lack of oxygen in the closed cells in which the spores were isolated, due to the presence of motile Monads which originated from resting cells isolated with the spores. But finally, at Dr. Loeb's suggestion, some fresh-water algæ were mixed with the spores as soon as they were returned to the temperature of the room. In this way a supply of oxygen was maintained, and the development of the spores began at once. The first change that can be observed is the appearance of a thin layer of protoplasm which grows out of the spore. This protoplasmic layer develops into a small spherical cell, which gradually becomes separated from the

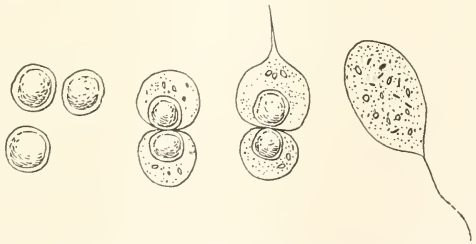


FIG. 4. Four stages in the development of the spore into the motile *Monas*.

spores. The cell at first is hardly visible because of the extreme transparency of its protoplasm, but it eventually becomes granular and develops a flagellum, which appears to originate as a long pseudopod extending outward from the protoplasm. The Monad formed in this way finally becomes motile and

swims away, leaving the empty spore behind. In some cases the spores are not discharged from the resting cell as soon as they are formed, but develope within its walls, so that it is possible to find spores in all stages of development, even the young Monads, within the old resting cell. The development of the spores usually occupies from two to four days, although in some cases it may be delayed for a much longer time. The Monads formed from the spores are at first spherical, and swim about with the characteristic revolving movement of this species of Monas. The cells rapidly become elongated, however, and within a short time are indistinguishable in all respects from the adult organism.

FIG. 5. Young Monads developed from the spores within the old cell wall of the resting cell.

A few typical experiments will be briefly described, as follows :—

Experiment I.—On April 11 a culture of *Monas* was placed in the refrigerator at a temperature of 1° to 4°C. The next day examination showed that all the Monads had formed spherical resting cells. Some of these were removed to the temperature of the room, and they immediately developed into the motile form. On April 24 many of the resting cells had broken up into spores. A small number of these resting cells and spores were isolated under the microscope. The resting cells immediately developed into the motile Monads as before. On April 26 a few of the spores had formed small Monads, but the development did not go very far because of a lack of oxygen in the closed cells which contained the spores.

Experiment II.—On April 18 a culture of *Monas* was put on ice at a temperature of 1° C. On May 3 the culture was removed to a temperature of 6° C. A large number of spores had been formed, a large proportion of them remaining within the resting cells. On May 6 many of the spores had developed into young Monads. A large number of the Monads were found swimming about within the walls of the resting cells.

Experiment III.—On May 14 a culture of *Monas* was placed on ice at a temperature of 1° C. On May 22 a large number of the resting cells and spores were removed to the room temperature and isolated under the microscope in closed cells that contained fresh-water algæ. Development of the spores commenced at once, and on May 25 they had reached the motile Monad form.

Summary.

1. In *Monas* a reduction of the temperature brings about certain structural changes within the cell, that result in the formation of many small spores, each of which has the power of reproducing the organism.

2. It is thus possible by means of variations in the temperature to control the methods of reproduction in *Monas*. At a temperature of 20° C. *Monas* multiplies sexually and by simple fission. At a temperature of 1° to 4° C. reproduction by asexual spores takes place.

DEGENERATION PHENOMENA IN THE LARVÆ OF GONIONEMA.

HENRY FARNHAM PERKINS.

Every student of invertebrate embryology has experienced the difficulty of rearing eggs and larvæ of marine animals with a view to determining the stages in the life-history of the species. One of the chief contingencies to be encountered is that which arises from the necessity of supplying an artificial environment which approaches closely enough to the natural conditions to permit the creatures to live and thrive in captivity, and to develop normally. In the effort to secure these favorable conditions many failures are to be expected, and it frequently happens that remarkable monstrosities are produced by some untoward condition which can frequently be only guessed at. Interesting and significant tendencies are sometimes to be traced in these abnormal forms, and light is thrown upon the normal constitution of the tissues and their power of assuming a variety of forms.

The transforming and regenerative power which is displayed by cœlenterate tissues has been adequately demonstrated by investigators in various orders. Among the hydroids, several instances have been recorded in which the soft parts of the colonies exhibit amœbiform activity. In *Hydra*, when live specimens are mounted in water under a cover-glass, so that the tentacles are in contact with the glass, their tips are frequently seen to become spread out in a smear against the glass, and flowing movement is observable in the protoplasmic tissue-layers. It may be added that the same activity occurs in the tips of the tentacles in the *Hydra*-like polyps of *Gonionema*, which furnish the data for the present paper.

In *Plumularia cristata* the soft internal substance of the colony, the sarcode, flows out of the vase-like nematophores which are borne upon the ramuli of the zooids, and after being protruded for a short distance send out long filamentous pseudopodia; these frequently branch and anastomose, like the pseudopodia of the amœba. The processes are afterwards withdrawn again into

the nematophores. An account of this process was first given by Allman.[1]

In the germ-plasm and ovarian eggs of certain members of the Hydrozoa, *Cunina* for example, amœbiform activities commonly occur. Metschnikoff[2] has described and figured many of these.

It will be remarked that these instances of amœbiform activities are all limited to portions of the individuals or to the earliest stages of unfertilized eggs. Dr. Loeb[3] found in *Campanularia* that when parts of a colony were brought in contact with a solid object such as the bottom of a dish the hydranths in that part degenerated and the zooids were changed into soft flowing protoplasm. The entire individual was involved in this change. The epiderm of the hydranths affected was left quite empty of sarcode, this being used in formation of stolons at other points of the colony.

The condition in *Gonionema*, also is one of complete modification. The animal undergoes a decided change, losing its characteristic shape and appearance, and becoming transformed into a simple multinucleate plasmodium which sometimes exhibits amœboid movements.

The simplest case of this manifestation of the amœba-like character of the tissues appears in the tendency to fusion, seen in the young polyps of *Gonionema*. This takes place in the hydra-like larvæ which develop from the free-swimming planulæ; these latter have a way of settling down in clusters on the bottom of the vessel in which they were reared, and it is a common thing to see two or three of these larvæ quite fused together at the base, so that they appear to be double- or triple-headed monsters. While such larvæ frequently live for some time, I have never happened to see any in an advanced stage of growth; it is possible that the condition is degenerative, and that it is the result of defective oxidation.

[1] Allman, J. G., 1884., "Amœboid Protoplasm in Hydroida," *Ann. and Mag. Nat. Hist.*, 3 13.

[2] Metschnikoff, E., 1874, "Embryologische Studien," p. 110.

amœboid cells.

[3] Loeb, Jacques, "On the Transformation and Regeneration of Organs." *Am. Jour. Physiology*, June, 1900.

A degenerate condition which is by far more striking and significant was observed in the case of individual polyps, which underwent fundamental changes of a remarkable character. The polyps in which these phenomena appeared were reared in the laboratory from eggs laid in August, 1901, at Woods Holl. Several lots of larvæ were reared in aquarium jars, some living for several months, others dying and quickly vanishing after a few weeks. Only one jar of larvæ exhibited the peculiar forms which are described in this paper. In all the jars, as nearly as possible, the natural conditions of the larvæ were imitated by keeping fresh algæ, ulva, diatoms, etc., in the water with the creatures. Some of the jars of polyps were left at Woods Holl for three months, and then forwarded to the zoological laboratory at Baltimore. These were found to contain healthy larvæ, which remained alive until February. But one of the jars, instead of remaining at Woods Holl for the balance of the summer, was taken directly to the laboratory, and the contents were frequently examined. About the first of October, the amœba-like forms were first seen. For some time no attention was paid to them, as they were simply taken for amœbæ which had been introduced with the sea water. But it was soon seen that the number of healthy polyps was rapidly diminishing, while the supposed amœbæ were becoming surprisingly numerous. More careful attention was then paid to the creatures, and it was discovered that the polyps were undergoing remarkable changes. Single individuals were noted from time to time, and observed to contract, first losing the *Hydra*-like aspect, the tentacles being completely retracted. The upright body of the polyp then settled down upon the glass, and all semblance of its natural shape was completely lost. Moreover, the differentiation between ectoderm and endoderm could no longer be discerned, except dimly in a few instances (*v*, Fig. 4). The cell-walls lost their distinctness and soon disappeared. In this remarkable way the originally vigorous hydra, standing upright with mouth gaping and tentacles widely extended, became quickly degenerated into a shapeless mass of substance without organs, only sluggishly motile, without discernible cell-boundaries or tissue layers, in short, an organism which was hardly more than a plasmodium.

In this condition the tissue of the creature was yellowish and translucent, with many inclusions of various sizes and shapes. The nuclei of the polyp were permanently visible, scattered unevenly through the substance. Vacuoles of varying size occurred in most cases, and a large number of foreign objects, such as bits of diatoms and disintegrating vegetable matter, particles of sand, etc., lay in the protoplasmic matrix.

This plasmodium showed most remarkable ability to move and change its form. From a regular mass of protoplasmic tissue long pseudopodia-like processes were seen to push out into the water or along the bottom. The creature had the power of flowing about on the bottom like an amœba, and its changes

FIG. 1.

of form were sufficiently rapid to be readily noticeable. No definite type of form could be discovered, the changes being apparently endless in diversity. Several figures are given to show some of the forms which appeared in the course of my observations; these are merely typical, and do not represent a tithe of the peculiar appearances which came to my notice. All the shapes shown in the figures were such as single individuals were frequently seen to go through within a couple of hours' time.

Starting, perhaps, as a spheroidal mass adhering to the bottom by a broad, sole-like surface (Fig. 1), in which condition the creature may have been resting motionless for some time, the form was suddenly seen to show signs of life, and to elongate vertically, so as to send upward into the water a columnar process (Fig. 2). This grew rapidly more slender at the base while the free end enlarged into an irregular bolt (Fig. 3). Usually the

surface of the larvæ was seen to be covered with a number of thorn-like points, which were evidently the ectodermal cnidocils of the polyp (Fig. 1). There was no sign of the nematocysts, these

FIG. 2.

having disappeared early in the degeneration of the tissues. The permanence of the cnidocils is an interesting point in the degen-

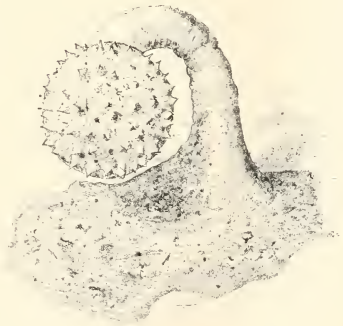


FIG. 3.

erative changes through which these larvæ were seen to pass. It gives a suggestion of the degree to which the tissue is stiffened and specialized to form these trigger-organs. From time to time

the outlines of the degenerated polyps, as seen in profile against the light, appeared smooth and devoid of cnidocils (Figs. 3 and 5.) But these same individuals soon changed their position in such a way as to display the full battery of trigger-organs, showing that the reason for the temporary disappearance was the flowing of the soft tissues, which caused the cnidocils to be engulfed in the substance, from which they were afterwards pushed outwards again to the surface. In the ball at the extremity of the columnar prolongation shown in Fig. 3 the cnidocils had temporarily disappeared, but with the subsequent changes of this individual they soon became visible again and remained so for a long time. The rod of tissue connecting this ball with the basal portion of the polyp became longer and more slender, and the mass at the tip assumed a more nearly spherical contour, as in Fig. 4. Following this appearance a change

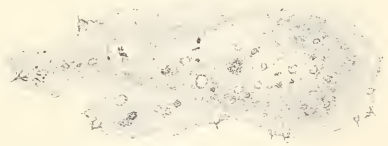


FIG. 4.

took place which was frequently manifested by the degenerating creatures and which ultimately brought about their disintegration. The ball at the tip of the rod-like process became constricted off and fell to the ground, there to undergo the same series of changes which characterized the previous history of the complete individual. The basal portion of the polyp, with the neck of tissue projecting from one part, became retracted into a more regular mass, and went on with its flowing movements and protrusion of pseudopodium-like processes. At first it was closely applied to the glass, and flattened at the edges (Fig. 5). It soon changed into the form shown in Fig. 6, which was the most common of all, and which was at first mistaken for an amœba. As the creatures moved slowly along the bottom, any minute particles of matter, organic or inorganic, which came in their path were engulfed by the protoplasm, and remained

there until ejected by the subsequent movement of the animal. It was not possible to determine whether these particles furnished any nutriment, but the long-continued activity of the degenerated polyps seemed to indicate their ability to absorb some food.

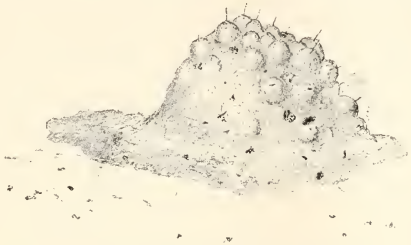


FIG. 5.

As the plasmodium crept along over the bottom it left in its wake a trail of particles which it had egested or which had become detached from the substance of the protoplasm itself.

FIG. 6.

A form which was noticeable both for its remarkable appearance and its frequent occurrence is represented by Fig. 7. This was derived, in a comparatively short time, from such a form as that of Fig. 6, a long filamentous process being sent upwards into the water, free from the bottom. Along this process the

substance of the main mass flowed out gradually until it had accumulated as a spherical knob at its extremity. On the surface of this knob the cnidocils protruded in all directions. A second filamentous rod of tissue was projected from another portion of the mass, and a similar flowing movement of the substance resulted in another knob. This waving object then proceeded

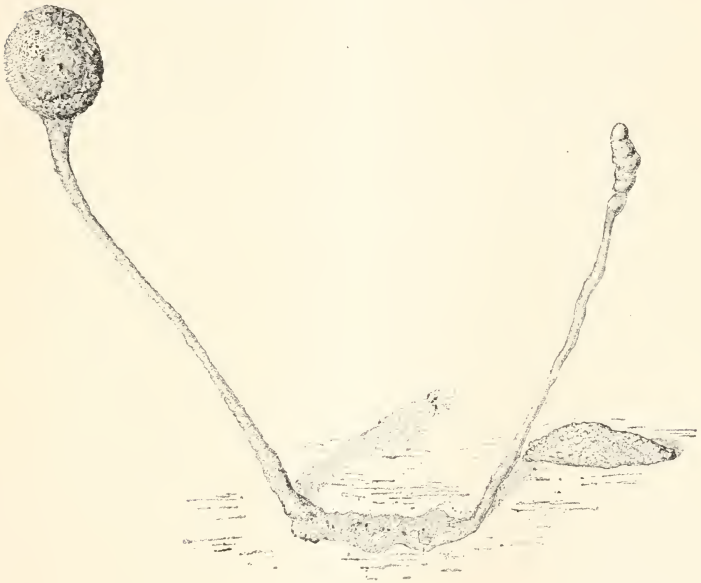


FIG. 7.

to crawl slowly over the bottom, the basal portion only exhibiting activity, and that the simplest. This form was frequently present in the dishes, as many as half a dozen specimens, more or less like Fig. 7, sometimes occurring within an area of not more than four square millimeters. By the constriction of the connecting rod at a point near the ball at its extremity, this latter mass was detached from the rest of the object, and settling down on the bottom began to move about in the characteristic way The remaining stock of protoplasm, seen on the right in the

figure, shortened and shrivelled (Fig. 7). This form arises without the slightest traceable connection with the polyp-form, and its appearance seems entirely fortuitous. It is hard to see why it should be so common.

The plasmodium-like larvæ remained alive for nearly two months, showing the various forms and movements described. The repeated fission of the individuals resulted in such diminution of the size of the pieces which came from the original individuals that after a time it was impossible to distinguish the bits of living matter from the other particles lying about on the bottom. But during the entire time in which it was possible to recognize the pieces of degenerating larvæ, by the evident cœlenterate characteristics, the sum total of this substance did not seem to be at all diminishing. It is impossible to assign any satisfactory explanation to the phenomena, but it is not unlikely that the condition of the water in this particular aquarium-jar was peculiar, owing, perhaps, to evaporation which increased the density, or to the introduction, inadvertently, of some chemical.

While it is not possible to explain the phenomena here described, or to determine their significance, the facts are stated in the hope that they may call forth other facts which may have a bearing on the same subject. It would be most interesting to discover whether such degenerative conditions occur in any other cœlenterates; whether they are brought about by any specific external circumstances; and whether creatures which have assumed this condition are capable of resuming their normal form.

Johns Hopkins University,
June, 1902.

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BIOLOGICAL BULLETIN.

AN AMERICAN CERAPACHYS, WITH REMARKS ON THE AFFINITIES OF THE CERAPACHYINÆ.[1]

WILLIAM MORTON WHEELER.

The singular ant described in the following pages was discovered by Miss Augusta Rucker within the city limits of Austin, Texas, on May 11 of the current year. Impressed by the peculiar, elongate, and segmentally constricted body of the insect, Miss Rucker fortunately secured nearly all the individuals in the nest, including the apterous queen. The most superficial study showed that the insect could belong to none of the described North or South American genera of Formicidæ. A note from Professor Emery, to whom a few of the specimens were sent, and comparison with some rare ants generously sent me by that gentleman and by Dr. Gustav Mayr, made it perfectly evident that the new form must be assigned to the interesting genus *Cerapachys* (subgenus *Parasyscia*), representatives of which were hitherto known only from the Old World (Africa, Asia and Papuasia).

The *Cerapachys* nest was found about six inches below the surface soil under a layer of large flakes of limestone in the shade of some hackberry trees growing on the banks of Waller Creek. At first only six workers and the queen were taken, but on the following day Miss Rucker and myself succeeded in unearthing four more workers on the same spot. The whole nest could not have contained much more than a dozen specimens. Unfortunately there were no larvæ or pupæ. When first seen the *Cerapachys* were engaged in a battle with some much smaller subterranean ants (*Ponera trigona* var. *opacior*) which seemed to be invading the nest. The new species was slow in its move-

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 37.

ments as would be expected in creatures with such long bodies and short legs. They did not "feign death" when roughly handled, though some of them, when held in the forceps, remained motionless in the attitude probably assumed when they are being deported by their sister workers. While creeping about the ants carried their very robust antennæ in a peculiar manner. The scape was held erect or inclined forwards, but hardly in a horizontal or lateral position as in other ants, while the funicle could be folded down onto the front surface of the scape a little obliquely to the side. The habits, so far as these could be inferred from the little I saw of the ants in a living condition, recalled those of *Stigmatomma pallipes* described in a former paper ('00). As the workers are quite blind it seems probable that the species leads a subterranean life, seeking its prey in the soil or under the dead leaves. This seems also to be indicated by the depth at which these small insects were found. The very robust antennæ and the beautifully developed stridulatory apparatus, which occupies the whole of the large membrane between the postpetiolar and first gastric segment, indicate that the senses of contact-odor and hearing are highly developed and may adequately compensate for the absence of visual organs.

On the 22d of May, Mr. C. T. Brues found a solitary worker of the same *Cerapachys* under a stone at Pease Park, about two miles from the locality in which the species was first taken. That the insect is extremely rare is shown by the fact that it had not been found before during three years of careful collecting in the vicinity of Austin.

CERAPACHYS (PARASYSCIA) AUGUSTÆ, sp. nov.

Worker (Fig. 1).—Length 2.5–3.5 mm. Head longer than broad, marginate and broadly excised behind and produced posteroinferiorly to form two acute, somewhat divergent angles, so that the head resembles in shape that of *Eciton schmitti* Emery. These posterior angles are continued downwards on either side as a fold which meets its fellow from the opposite side on the lower posterior surface of the head. Sides of head faintly and evenly convex; eyes entirely absent; lateral carinæ well-developed; frontal carinæ high, projecting, closely approximated, extending a short distance back between the antennal foveæ and ending on either side in a distinct tooth just in front of the rather pronounced frontal depression. Mandibles triangular, curved downwards at their tips, with

distinctly crenated edges to their blades. Antennal scape somewhat more than half the length of the head exclusive of the mandibles, rapidly incrassated towards its apex, which is provided with a deep concavity on the anterior lateral surface for the insertion of the funicle ; funicle 10-jointed ; first joint longer than broad, almost concealed in the concavity of the scape ; joints 2-9 distinctly broader than long, gradually increasing in size distally, terminal joint very large, glandiform ; constituting a club which is as long as the five preceding joints of the funicle. Thorax cylindrical, fully two and one half times as long as broad, oblong when seen from above, dorsal surface flattened, mesoëpinotal suture hardly indicated by a faint constriction. Posterior surface of epinotum abruptly declivous, carinate on either side and with an indistinct tooth above. Petiole subcuboidal, a little longer than broad, with flat dorsal surface ; lower surface produced anteriorly into a large, compressed, plowshare-like tooth. Postpetiole flattened dorsally, one and a half times as long as the petiole ; when seen from above its anterior margin is hardly broader than the petiole, but its posterior border is half again as wide ; its lower surface is convex and projects forward a little in front of the anterior dorsal border. Stridu-

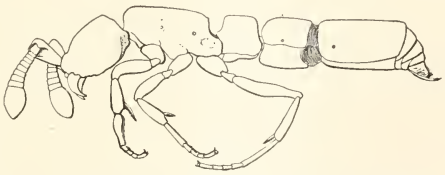


FIG. 1. *Cerapachys* (*Paravyscia*) *augustæ* n. sp. Worker.

latory organ highly developed, occupying the whole of the intersegmental membrane between the postpetiole and gaster. First gastric segment cylindrical, flattened on its dorsal surface, fully one and a half times as long as the postpetiole, slightly wider behind than in front. Remaining gastric segments very short, forming a rapidly declivous termination to the abdomen ; second, third and fourth gastric segments of about equal length, tergite of the fifth segment triangular, covered with small but distinct spines on its lateral and posterior border. Sting thick, exserted. Legs rather short, all the tibiæ furnished with pectinate spurs.

Surface of body shining, except the head, which is subopaque. Mandibles finely and indistinctly striated. Head covered with large, close-set, umbilicate foveolæ except on the folds of the posterior angles which are coarsely coriaceous. Whole thorax covered with umbilicate foveolæ like those on the head. On the petiole and postpetiole the foveolæ are as large as those on the head and thorax but less densely aggregated ; on the first gastric segment the foveolæ are distinctly smaller and much further apart.

Whole body covered with long, suberect, golden yellow hairs, which on the head, thorax and abdomen, arise from the umbilicate centers of the foveolæ. Hairs on the terminal antennal joint very short and dense, contrasting with the longer hairs on the scape and short joints of the funicle.

Color red, edges of mandibles, clypeus, anterior border and posterior angles of head, the funicle with the exception of the terminal joint, the articulations of the thorax, legs and abdomen and the tip of the latter blackish. Legs and terminal antennal joint slightly more yellowish than the remainder of the body.

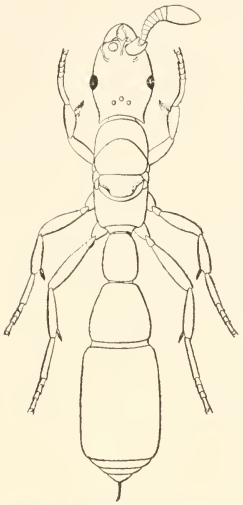


FIG. 2. *Cerapachys* (*Parasyscia*) *augustæ* n. sp. Apterous female.

Female (Fig. 2).—Length 3.75 mm.

Eyes moderately large, convex, situated in the middle of the lateral surface of the head, which is shaped like that of the worker. Ocelli well developed, not lying at the corners of an equilateral, but of an isosceles triangle with a long base. Prothorax large, scutum of mesonotum well developed, dorsally flattened, without parapsidal sutures; tegulæ large, elliptical; no paraptera between the scutum and the well-developed, flattened scutellum, metanotum narrow but distinct; epinotum large, shaped like that of the worker. On the pleural surface the mesothoracic epimerite and episternum are distinct but these elements in the metapleuræ are more obscurely separated. There is nothing to show that the thorax has ever borne wings. Petiole longer than broad, postpetiole almost twice as broad as the petiole, its posterior border nearly coëxtensive with the edge of the first gastric segment, which is both broader and longer than this segment in the worker. Terminal gastric segments and sting in all respects like those of the worker.

The sutures of the thoracic dorsum are blackened; otherwise the female is like the worker in coloration, sculpture and pilosity.

As *Cerapachys augustæ* has 11-jointed antennæ it must be placed in Emery's subgenus *Parasyscia*. Emery ('01[a] and '01[b]) has recently published a revision of the ants of the subgenus *Cerapachys* and the allied genera which he groups together as Cerapachyinæ, a supertribal division comprising the following tribes, genera and subgenera.

Tribe 1. Acanthostichii—with the single South American genus *Acanthostichus* Mayr.

Tribe 2. Cerapachyi (Africa, Asia, Australasia and Texas!).

Genus *Cerapachys* Smith (antennæ with a one-jointed club).

Subgenus *Cerapachys* (antennæ 12-jointed).

Subgenus *Parasyscia* Emery (antennæ 11-jointed).

Subgenus *Oöcræa* Roger (antennæ 10-jointed).

Subgenus *Syscia* Roger (antennæ 9-jointed; first gastric segment but little longer than the postpetiole).

Subgenus *Cysias* Emery (antennæ 9-jointed; first gastric segment much longer than the postpetiole).

Genus *Phyracaces* Emery (terminal antennal joint not forming a club — Madagascar, Africa, Borneo).

Genus *Lioponera* Mayr (2–3 terminal joints of antenna forming a club — India, Sumatra).

Genus *Sphinctomyrmex* Mayr (abdomen constricted behind each segment — Brazil).

Tribe 3. Cylindromyrmii.

Genus *Cylindromyrmex* Mayr (antennæ 12-jointed; South America).

Genus *Simopone* Forel (antennæ 11-jointed; Madagascar).

The occurrence in America of a representative of the largest and most diversified genus of the Cerapachyinæ is of some interest in view of the fact that this group is the most archaic and generalized of existing Formicidæ. It is, in fact, the group from which Emery and Forel would derive both the Dorylinæ and Ponerinæ, themselves very primitive subfamilies of ants. Inasmuch as the three remaining subfamilies (Myrmicinæ, Dolichoderinæ and Camponotinæ) are derivable from Ponerine forms, it is evident that the Cerapachyinæ must constitute a group of high phyletic significance. Emery ('95) has even gone a step further and pointed out the close resemblance of the Cerapachyi to the Mutillidæ, especially to forms like *Apterogyna*, which have a very ant-like pedicel to the abdomen and resemble the ants in many other particulars. I have copied his figure of *Apterogyna* (Fig. 5) for the sake of showing the close resemblance of this primitive Mutillid to certain species of *Cerapachys*, *e. g.*, *C. peringueyi* (Fig. 3).

While Emery and Forel agree in regarding the Cerapachyinæ as the most primitive of Formicidæ, they hold very different

opinions concerning the subfamily to which the group should be assigned. Emery ('95, '01[a]), who emphasizes morphological characters, regards the Cerapachyinæ as veritable Dorylinæ, while Forel ('99, '01), who is inclined to lay considerable stress on ethological characters, maintains that these ants are true Ponerinæ. Accordingly Emery includes the Cerapachyinæ and Dorylinæ (*sens. str.*) as two coördinate groups under the sub-

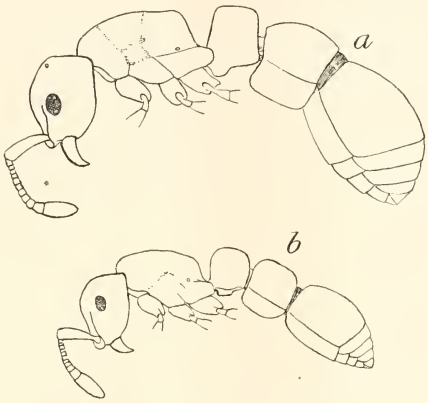


FIG. 3. *Cerapachys peringueyi* Emery. *a*, female (apterous); *b*, worker. After Emery.

family Dorylinæ, whereas Forel would regard the three tribes Acanthostichii, Cerapachyi and Cylindromyrmii as so many tribes of Ponerinæ.

Emery's position may be stated as follows: Almost nothing is known concerning the habits of the Cerapachyinæ and mere ethological inferences cannot help us in deciding the question of affinities. The female of *Acanthostichus* (see Fig. 4, *a*) is wingless and decidedly *Dichthadia*-form ("bâtie sur le type *Dichthadia* à peine mitigé"), like the females of *Dorylus* and *Eciton*. The males of the Cerapachyinæ have no cerci and have retractile copulatory organs like the Dorylinæ, whereas the cerci are present and the genitalia more or less exserted in the male Ponerinæ. In general, comparatively little value can be attached to the con-

ditions of the pedicel in the taxonomy of ants, so that we should not emphasize the Ponerine-like petiole and postpetiole of the Cerapachyinæ, especially as all sorts of pedicels are found among the different genera of this group from that of *Acanthostichus*, which is like *Amblyopone*, to that of *Oöcerœa*, which recalls the conditions seen in the Myrmicinæ. The larvæ of the Cera-

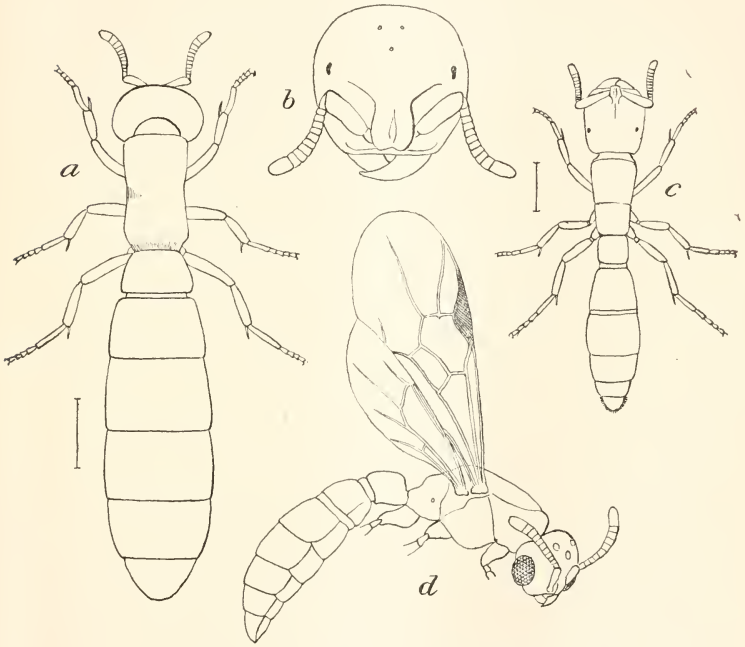


FIG. 4. *a*, *Acanthostichus quadratus*, ♀ ; *b*, head of same from front ; *c*, large worker (same magnification as *a*) ; *d*, *Acanthostichus fuscipennis*, ♂. After Emery.

pachyinæ are unknown, but as non-tuberculate larvæ, somewhat resembling those of *Eciton*, occur in some distinctively Ponerine genera (*Stigmatomma* and *Ectatomma*) it is probable that the larval characters would have little weight in solving the problem under consideration.

Forel, on the other hand, advances the following reasons for regarding the Cerapachyinæ as true Ponerinæ. While they undoubtedly exhibit traits which ally them with the Dorylinæ, their habitus is, nevertheless, decidedly Ponerine. The little that is known of their habits certainly indicates that they live in small, stationary colonies like the Ponerinæ, instead of populous, nomadic colonies like the Dorylinæ. The queens, moreover, are so nearly of the same size as the workers as to preclude anything like the great fecundity of the queens of *Dorylus* and *Eciton*. The Cerapachyinæ, too, have short legs of such a structure as to indicate a slow gait and more sedentary habits. The workers of the Cerapachyinæ have ordinary facetted eyes, whereas those of the Dorylinæ are absent or ocelliform, while the atrophied eyes of the Ponerinæ have a very different structure.[1] The conditions of the

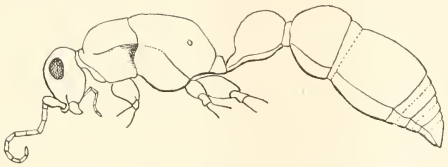


FIG. 5. *Apterogyna olivieri*. Female, after Emery.

pedicel in the males, females and workers of the Cerapachyinæ are correlated as they are in the Ponerinæ, and do not exhibit the differences seen in the Dorylinæ between the worker on the one hand and the male and female on the other. The wingless con-

[1] The distinction to which Forel calls attention is worthy of histological study. I am inclined to think, however, that it may be a distinction without a difference. I have recently sectioned a number of pupæ of *Eciton schmitti* and find that the ocelliform lateral eyes are really very much atrophied compound eyes, too much atrophied, in fact, to be at all functional as visual organs. *The retinal hypodermis, which is somewhat thickened under the convex lens, shows indistinct but unmistakable traces of ommatidia. The optic nerve is very short and not connected with the brain. It ends freely in a blunt point a short distance from the ommatidial layer. This is interesting as proving that the visual fibers must arise in the retina and grow towards the brain and not in the reverse direction from cells in that portion of the brain known as the optic ganglion.* If there is a distinction between the abortive eyes of the Dorylinæ and Ponerinæ it would seem to be that in the former subfamily the ommatidia disappear both by fusion with one another and by reduction in number, while in the latter the number of ommatidia is gradually reduced without fusion.

dition of the female Dorylinæ is not of so much value in view of the fact that some true Ponerinæ (*Leptogenys*, *e. g.*) have wingless ergatoid queens. The characters drawn from the male genitalia are not of themselves sufficiently important to determine the allocation of the Cerapachyinæ in a particular subfamily.

My own observations on the living *Cerapachys augustæ*, though very fragmentary, incline me to accept Forel's views and to regard the Cerapachyinæ as true Ponerinæ. There was certainly nothing in the habits of the insects to remind me of the Doryline ants notwithstanding their striking morphological resemblance, especially in the shape of the head, and the blindness of the workers, to certain species of *Eciton* (*E. schmitti* Emery, *E. sumichrasti* Norton, *E. wheeleri* Emery). The small colony, with its queen so like the workers in size and structure, and the slow movements of the insects, all very closely resemble the conditions found in many lowly organized Ponerinæ, *e. g.*, *Stigmatomma*, and I may add also, *Proceratium* and *Sysphincta* to judge from the account (*in litteris*) of my friend, Rev. P. J. Schmitt, O.S.B., who has been so fortunate as to observe living colonies of these rare ants. Moreover, the fact that specimens of Cerapachyinæ are rare in collections would seem to show that the nests of these insects are not at all populous. It is safe to say that if *C. augustæ* were like the timid *Ecitons* in its habits, it would have been impossible to find a remnant of the colony on the day following the ransacking of the nest. Morphologically the Ponerinæ are certainly a very heterogeneous assemblage of forms, but this is merely what we should expect to find in so ancient and extensive a group. The addition of the tribes of the Cerapachyinæ to this subfamily would increase but little the already existing heterogeneity, since these ants are closely related to forms like *Proceratium* and *Sysphincta* which Emery formerly regarded as Dorylinæ but has since placed with the Ponerinæ. When the Cerapachyinæ are included with the Ponerinæ, the Dorylinæ become a more homogeneous subfamily, while the Ponerinæ represent the diversified and often peculiarly specialized survivors of the ancient stock from which all the other subfamilies of ants have been descended.

It is an interesting and probably significant fact that all the

various forms of females to be met with among the Formicidæ are already foreshadowed in the small and very primitive group of the Cerapachyinæ. Though we have no knowledge of the females of several of the genera, we may recognize no less than four different female forms:

1. The female of *Acanthostichus*, which, as Emery has shown ('95), is decidedly *Dichthadia*-like, *i. e.*, unmistakably like the huge blind and wingless females of *Dorylus* and *Eciton*. This female is considerably larger than the largest workers of the colony as shown in Emery's figures of *A. quadratus* which I have reproduced in outline (Fig. 4, *a*, *b* and *c*).

2. Normal winged females like those of most genera of Formicidæ but more similar to the workers in size and structure. These females are known to occur in the genera *Lioponera*, *Cerapachys* and *Sphinctomyrmex*.

3. The female of *Cerapachys peringueyi* from South Africa (Fig. 3, *a*). According to Emery ('95) this form is wingless and not much larger than the worker (Fig. 3, *b*), which it closely resembles in structure. It may be designated as an ergatoid female and is not unlike the ergatoids occasionally found in species of *Ponera* (*P. coarctata*, *P. opaciceps*, etc.).

4. The female represented by the above described *Cerapachys augustæ* from Texas. This form is wingless but in thoracic structure resembles the winged females of the Ponerinæ in general. It is but little larger than the largest workers though possessing well-developed eyes and ocelli.

This "morphological restlessness" in the structure of the females of so small a group of genera as the Cerapachyinae is, perhaps, significant as the phyletic source to which the different female forms of all the subfamilies of ants are to be traced. We may look upon the *Dichthadia*-like queens of the Dorylinæ as a further development of the conditions exhibited by *Acanthostichus*, and the ergatoids, which crop out sporadically among the Ponerinæ and the higher subfamilies, may, perhaps, be regarded as cases of reversion to females of the type of *Cerapachys peringueyi* and the Mutillidæ (*Apterogyna*, *e. g.*). The pseudogynic forms of the more specialized ants (*Formica*, *Camponotus*) resemble the female of *C. augustæ*. Finally, the winged females

of *Lioponera*, *Sphinctomyrmex*, etc., like the normal winged females of the majority of the Ponerinæ, lead very naturally to the conditions seen in the more modified winged females of the Myrmicinæ, Dolichoderinæ and Camponotinæ.

BIBLIOGRAPHY.

Emery, C.

'95 Die Gattung Dorylus Fab. und die systematische Eintheilung der Formiciden. Zool. Jahrb. (Abth. f. Syst.), 8 Bd., 1895, pp. 686–778. Taf. 14–17, 41 text-figs.

'01a Notes sur les Sous-familles des Dorylines et Ponerines (Famille des Formicides). Ann. Soc. Ent. Belg., Tome XLV., 1901, pp. 32–54.

'01b Note mirmecologiche. I. Revisione del Gruppo dei Generi Affini a Cerapachys F. Sm. Rend. Sess.R. Accad. Sci. Ist. Bologna, Anno 1901, 8 pp.

Forel, A.

'99 Formicidæ (in Biologia Centrali-Americana), 1899, pp. 2 and 22.

'01 À propos de la Classification des Fourmis. Ann. Soc. Ent. Belg., Tome XLV., 1901, pp. 136–141.

Wheeler, W. M.

'00 The Habits of *Ponera* and *Stigmatomma*. Biol. Bull., Vol. II., No. 2, Nov., 1900.

ROCKFORD, ILLINOIS, July 6, 1902.

STUDIES ON THE LIFE-HISTORY OF PROTOZOA. III, THE SIX HUNDRED AND TWENTIETH GENERATION OF PARAMŒCIUM CAUDATUM.[1]

GARY N. CALKINS.

Over 65 years ago, Ehrenberg came to the conclusion that the Protozoa are potentially immortal. Having all of the necessary organs and losing nothing in reproduction, there was no reason at all, he argued, why they can not live indefinitely. Shortly after this, Dujardin published a work in which he stated that Protozoa have periods of activity alternating with periods of rest, and Ehrenberg's view was vigorously contested.

Among contemporaneous biologists, the matter has been taken up, on the one hand, by Weismann and his followers, on the other by Maupas and those who see in Maupas' work the best weapon with which to combat Weismann. The matter at issue involves an interesting question, *viz.*, will protoplasm having every opportunity to exercise its various activities, and without being specialized through differentiation to the performance of limited functions, continue to live indefinitely, or will it gradually become exhausted and die as do the multicellular animals? Weismann, following the line of reasoning pointed out by Ehrenberg, maintains the former point of view, while Maupas, on the other hand, upholds the latter and argues, that, as the race becomes older, the vitality becomes more and more reduced, until finally, the Protozoön, like the Metazoön, dies from old age. Both writers have had to take into consideration the factor of conjugation with the accompanying phenomena which were almost entirely unknown to the earlier controversialists. With Weismann, conjugation is merely one of the vital functions of protoplasm, to be considered in the same light as feeding or excreting and not in any way interfering with the theory of immortality. Maupas

[1] A lecture delivered at the Marine Biological Laboratory, Woods Holl, Mass., July 28, 1902.

argues that, like Metazoa, the race would show unmistakable signs of senile degeneration and would die of old age were it not for conjugation which, in some unknown way, restores the vitality and ensures a new cycle of generations. According to Maupas, therefore, it would seem that the protoplasm of the unicellular organisms is not capable of the perpetual exercise of the ordinary vital functions. His conclusions lead to the assumption that, after conjugation, the individuals have a certain potential of vitality which is gradually used up in the course of a more or less definite number of generations, and which can be restored again and again by conjugation.

The general theories involved in this discussion seem to me important enough to warrant further experimental tests, and with this in mind, I started the culture of two different lines of *Paramœcium caudatum*, on the 1st of February, 1901, and these have been under continual observation ever since. One of the lines died out last month, in the 570th generation, the other is still living in the 665th generation.

One definite aim at the outset was to ascertain whether a given form under culture would pass through a more or less clearly-marked cycle of activity to die ultimately of old age as Maupas maintained. Another aim was to find out whether there is a well-marked degeneration of different parts of the cell as age advances. These aims merely called for the repetition of Maupas' work in the case of one organism and were interesting enough in themselves. There was still another aim, however, which lent added zest to the work and made every stage one of fascination. This aim was to find out, if possible, the significance of conjugation and why it is that vitality is renewed by this means. It seemed to me that if Professor Loeb could induce artificial fertilization in the echinoderm egg, it should be possible to induce artificial rejuvenescence in *Paramœcium*. The first definite problem that suggested itself was the discovery of some substitute for conjugation that could be used when the periods of physiological depression were imminent. The experiments have justified the assumption that such substitutes would prove successful, for I have been able to carry these minute organisms, not through merely one period of so-called degeneration, but

through five such periods, and have been able to rejuvenate them "parthenogenetically" five successive times. I feel that I am now in the position to confirm the early observation of Dujardin that, in *Paramœcium* at least, there are more or less regular periods of vigor and depression which alternate with each other in fairly regular succession. At each period of depression, the organisms, had they not been stimulated in some way, would have died, thus showing that they were physiologically worn out. The subtitle of this paper is taken from one of the periods of depression when the last survivors of a long line were nearly lost.

The methods employed in the investigation are practically the same as those used by Maupas. The infusoria are kept in cells in depression slides filled with a food medium of hay-infusion. The original *Paramœcium* of the A series was taken from the pond water of Van Cortland Park, New York, and transferred to one of these cells. In two days it had divided three times and of the resulting eight individuals four were isolated in the same way as the first had been, while the remainder were set aside as "stock." The four lines thus started have been maintained throughout and give a better basis for the study of vitality of the race than one line could possibly have given. A second series—B—was started at the same time with an individual *Paramœcium* from Fort Lee, New Jersey, and four lines of this series were likewise carried on with the A series. Individuals are isolated every one or two generations and the records are kept in the form of a table showing the number of generations since the last isolation and the total number since the beginning.

The division-rate is taken as the measure of vitality, for it represents the rate of metabolism, growth and reproduction. A better index of the general vitality could not be found and while fairly constant from day to day, its fluctuations mark out clearly the periods of vigor and depression. The variations of the rate are difficult to follow when the daily records alone are considered and the cultures had been under way for ten months before a method was discovered of representing it graphically. When the daily rate of division is plotted the fluctuations of the curve, due partly to varying temperature and the food conditions, are perplexing and it is extremely difficult to compare the con-

dition of the general vitality at different periods by this means. Good results were finally obtained by averaging the number of divisions per day for definite periods, and plotting the averages. By this method of illustration the condition of the general vitality at any period desired can be seen at a glance. (See Fig. 1.)

The division-rate, as represented by such a curve, continued without noticeable variations from the beginning of the experiments until the latter part of the following July. About the 20th of that month the organisms began to die off at an alarming rate, indicating that a period of depression had apparently set in, or "degeneration" in the Maupas sense. Now was the time, if at all, to experiment with substitutes for conjugation, and in conformity with my original plan, the first trials were made with a change of diet. The organisms had been living steadily on the bacteria that develop in hay-infusion (*Bacillus subtilis* mainly), and various substitutes were now tried. Vegetable infusions of different kinds gave no improvement but meat infusions proved successful. The first experiment with the latter was with teazed liver which was added to the usual hay-infusion. The result was very gratifying for the organisms began immediately to grow and to divide, the rate of division rising from 5 to 9 divisions in successive 10-day periods. The effect of the liver was not lasting, however, and in the following 10-day period, the rate fell off to one division in five days or two-tenths of a division per day. During this time the organisms had not been continuously on liver treatment but had been transferred to the usual hay-infusion diet after 48 hours in the liver medium. At the end of the first 10-day period in August the cultures were again dying off, this time more rapidly than before and the renewed use of teazed liver had absolutely no effect. One thing after another was now tried without success, the organisms meanwhile growing weaker and weaker. Finally they were transferred to the clear extract of lean beef made by boiling fine pieces of beef in tap water. The effect of this medium was interesting, for, although it restored the weakened vitality, there was no rapid increase in the rate of division as when first treated with the teazed liver. The infusoria were, however, now large and vigorous and did not die unless transferred from the beef medium to the usual hay infusion. When this was attempted,

they would become abnormally active and would finally die. The division-rate gradually increased during the month of August until, in the last ten days, they averaged six generations. Finally, in September, the attempts to get them back on the old diet of hay-infusion were successful, and then the division-rate went up at once to twelve times in ten days, and a month later, they were dividing at the rate of fifty times a month.

This experience showed that "rejuvenescence" in the Maupas sense, can be brought about without the aid of conjugation. Had it not been for the extract of beef, or some other equally beneficial substance, all of the organisms would have died, and, like Maupas,[1] Joukowsky[2] and Simpson,[3] I should have assumed that their possibilities of continued living had been exhausted and that they had died from "old age" in the 198th generation.

From November 1st to December 10th the curve shows that the division-rate and the general vitality gradually decreased, and by the middle of December, a similar experience to that of midsummer threatened the cultures. Profiting by that experience I took the precaution of giving some of the individuals beef-extract before they got too weak, while others were continued on the hay-infusion. All of the latter died within a few days. Those that had been fed with beef-extract for 48 hours continued to live when put back in the hay-infusion, and they continued through another cycle of almost three months without treatment again with the beef.

From the middle of December on, the cultures were continued under slightly different treatment. Two of the four lines of As (A–1 and A–2) and two of the four lines of Bs (B–1 and B–2), were treated once per week for 24 hours with beef extract. This treatment was continued until the middle of March, when they were given hay-infusion without further variation. The other lines of As and Bs, *viz.*, A–3, A–4, and B–3, and B–4, were treated in December with the beef-extract and then kept on hay-infusion without further change. By this treatment it was possible to compare the general vitality under conditions of a more or less

[1] Maupas, E., *Archives d. Zoöl. expér. e. gén.* (2), VI. and VII., 1888, 1889.
[2] Joukowsky, D., *Verh. nat. Med. Ver. Heidelberg*, 1898, Bd. XXVI.
[3] Simpson, J. Y., *Proc. Roy. Soc. Edinburgh*, 1901.

regular stimulus, with the general vitality under conditions of an initial stimulus. The history of these experiments is shown graphically in the diagram shown on page 198.

On the first of January, all were in fairly good condition and the division-rate was about the same for all. All showed a decline in the latter part of February, and the rate of division rose for all of them in March. At this time there was a sudden warm period in which the temperature for that ten-day period increased 3 degrees on the average and this apparently acted as a mild stimulus. From this time on, however, the division-rate of the hay-fed individuals began to fall, and as soon as this fact was noticed, the beef treatment of the others was stopped. The hay-fed individuals did not recover their vitality and their division-rate continued to fall until finally stock and all died, apparently worn out. The beef-fed individuals, on the other hand, continued their high rate of division throughout the period of decline of their sister-cells, and did not show signs of diminished vitality until the first period in June. At this time, however, the rate fell very rapidly and the depression was clearly apparent in the daily records (See Fig. 1). The organisms at this time had not had beef-extract since March and they were accordingly transferred to it now with perfect confidence on my part that it would again prove to be the "elixir of life" for the *Paramœcium. This time, however, it failed to give the looked-for result, and the animals continued to die at a rapid rate.* Their appearance indicated that their trouble was not the same as in the previous periods of depression. Hitherto at such times the endoplasm appeared black under the microscope from the large numbers of gastric vacuoles with their food contents unchanged. At this time, on the other hand, the endoplasm was clear and free from such food masses, but the appearance of the protoplasm was singularly granular and different from that of a healthy individual. All changes that had proved beneficial before were now tried. These included not only liver, various vegetable infusions and the like, but salts of various kinds. Nothing seemed suitable for the organisms and they died off at a faster rate than ever. *When the last of the B series, stock and all, died in the 570th generation (June 16), and the A series became reduced to only 6 individuals in the 620th*

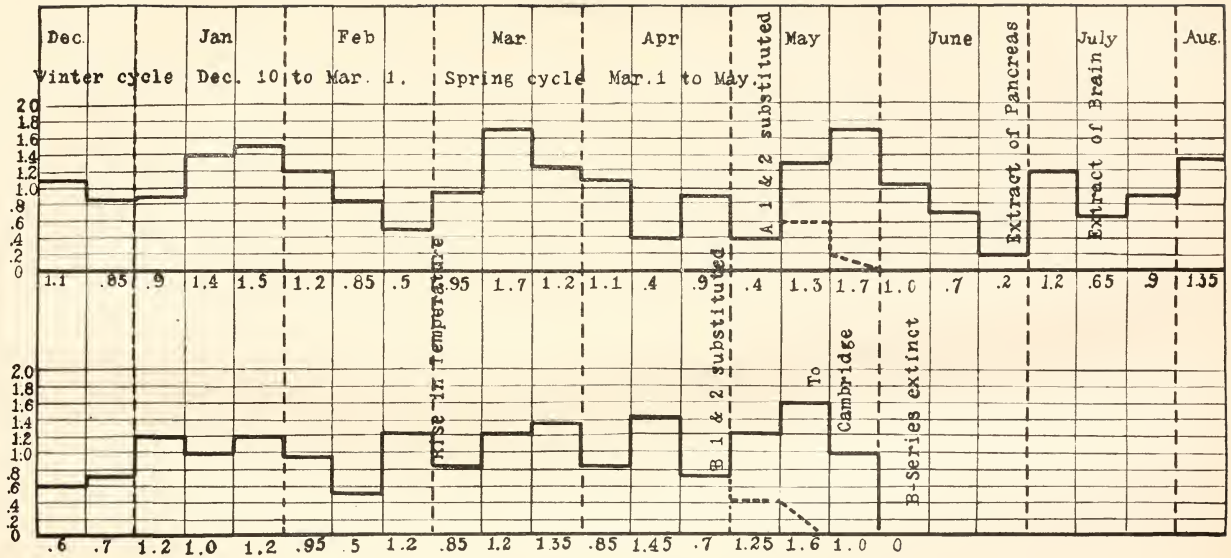


FIG. 1. Curve showing the history of the cultures from December 10, 1901, to August 10, 1902. The upper curve represents the A-series, the lower one, the B-series. In the former, the daily division-rates for A–3 and A–4 are averaged and these again are averaged for 10-day periods. In the latter, B–3 and B–4 are averaged in the same way. A–3, A–4, B–3 and B–4 were given beef extract in December and were then continued on hay-infusion until all four lines became extinct in May. At this time it became necessary to substitute A–1, A–2, B–1 and B–2 for the weakening sister lines whose further history is indicated by the dotted lines. The ones that were substituted had been fed once per week for 24-hour periods with beef-extract, but this treatment was stopped in March and they were given hay-infusion from that time on. The cycles thus overlap, for the beef-fed lines in March were in the same stage, as regards stimulation, as the hay-fed ones were in December. The B-series became extinct in June; the A-series were saved by change of diet. Those that were "rejuvenated" by extract of brain were substituted for those that were treated with extract of pancreas as soon as it was seen that the latter did not have a permanent effect.

generation (*June 27th*) *it looked as though the cultures were about to come to an end.* The unusual appearance of the organisms made me think that the trouble was not with the digestion of food, but with its assimilation, and with this clue in mind I made extracts of sheep's brain and sheep's pancreas. Three of the six remaining individuals were put into the brain-extract and three into the extract of pancreas. The latter worked best and the 621st generation appeared the next day. The former, while giving slower results, produced healthy and normal organisms, and in three days there were more than 30 individuals, and enough to experiment with. After 48 hours' treatment with each of these extracts the organisms of the regular series were transferred to the usual hay-infusion. Since then the division-rate has been fairly constant, and, as stated at the beginning of this paper, they are to-day (July 28th) in the 665th generation.

The effect of the two extracts was very interesting. The individuals that were treated with pancreas had been in N/25 magnesium chloride for thirty minutes on the sixth day of May. Those that were treated with the brain-extract had been in N/1250 potassium phosphate for thirty minutes on the eighth of June. The division-rate of the former, notwithstanding the quicker start, soon fell behind that of the latter, and to-day the only ones alive are the descendants of those that had been fed with the brain-extract.

The interpretation of these facts involves the interpretation of many of the deepest problems of biology. If we could point out their significance, it would mean that we are in a position to throw light upon the ultimate secrets of vitality; secrets which have taxed the ingenuity of philosophers of all ages, and which lie at the bottom of modern biological research. At the present time the question cannot be answered, and it seems mere presumption to attempt an explanation. Nevertheless, there are sufficient data to warrant a tentative interpretation even though it is only a vague, shadowy impression of what may actually go on in living protoplasm.

The bacteria in the hay-infusion constitute the normal food of the Paramœcidæ. Of these, *Bacillis subtilis*, is, probably, the only one left alive after the infusion is raised to the boiling point, and

this organism, therefore, forms the staple article of diet for *Paramœcium* in culture. In the body of the infusorian the bacteria are collected in the usual gastric vacuoles where the first stages, at least, of digestion are carried on. No one has identified the proteolytic ferment which is active in this process. The work of Meissner, LeDantec, Fabre-Domergue, Greenwood and others has shown that something analogous to pepsin plays the most important rôle in the process, but nothing definite concerning the ferment is known. In an allied group, however, the Rhizopoda, some work has recently been done which may throw light upon the digestive processes of the Infusoria. H. Mouton,[1] working in Metschnikoff's laboratory, has succeeded in extracting the proteolytic ferment from a bacillus-eating *Amœba*. Tests of various kinds showed that the ferment is more like trypsin in its action than like pepsin, and Mouton comes to the conclusion that it is intermediate between the two. As the foods of this *Amœba* and of *Paramœcium* are probably similar in proteid composition, it is not improbable that the proteolytic ferments are much alike.

Whether or not the proteolytic ferment in *Paramœcium* is the same as that in *Amœba*, there is no doubt that some such active agent is operative in the digestive processes of our infusorian. Furthermore, there is a certain amount of evidence to indicate that it is this ferment-forming power that becomes weakened in the protoplasm of *Paramœcium* as the race grows older. Physiological "degeneration" is not due, as Maupas supposed, to the loss of some of the necessary organs of the cell through degeneration, nor is it due to inability to take food, for, when in the depressed condition, the *Paramœcium* still maintains a current of bacteria into the mouth-opening, and food vacuoles are still formed. Frequently when in this condition the organisms appear black because of the undigested food balls, a fact which indicates that it is the digestive function that wears out. In this connection it is interesting to note the effect of dilute alcohol on the general metabolism of *Paramœcium*. Mr. C. C. Lieb, work-

[1] H. Mouton. Recherches sur la digestion chez les Amibes et sur leur diastase intracellulaire. Thesis presented at the Faculté de sciences de Paris, 1902. Reprint pp. 1–60.

ing with *Paramœcium* from these cultures, found that alcohol will increase the division-rate above the control, by about 33 per cent. Under the action of the alcohol the organisms are always lively and appear transparent as though all food were rapidly digested. The result is interpreted as a stimulus to the digestive processes effected by the alcohol.[1] In the period of depression which is represented by the 620th generation, alcohol brought about no stimulus and the organisms died as rapidly as the ones not thus treated.

As the age of the race increases, it is conceivable that something in the chemical make-up of the protoplasm becomes depleted, something which cannot be replaced from the constituents of the ordinary food medium. With the change of diet, however, new substances are introduced into the protoplasm and renewed activity results. Thus with the change from hay-infusion to beef-extract, chemical substances are introduced into the protoplasm which could not be obtained from the hay-infusion. In the New York water there are certain chlorides, nitrates, etc., all in extremely minute quantities and these are present of course in the hay-infusion. They are also present in the beef-extract which is made with the same water, but in addition to these salts there are certain extractives from the beef such as potassium phosphate and chloride, lactic acid and lactates, etc., substances of quite a different character from those of the ordinary medium. Added to the fact of a different chemical medium is the fact of a difference in osmotic pressure, for the extract is more dense than the hay-infusion. The extract does not have a direct stimulating effect upon the digestive processes and upon division, for, while the organisms are immersed in it, there is a very slow division rate; when transferred again to the hay-infusion, however, they divide more rapidly than before. From this it appears that the extract is not a food in the ordinary sense but that it is a stimulant acting through the salts dissolved in it.

These results justify, I believe, the conclusion that, as the potential of vitality becomes reduced, something in the chemical

[1] Cf. Calkins and Lieb. Studies on the Life-History of Protozoa. II., The Effect of Stimuli on the Life-Cycle of *Paramœcium caudatum*. *Arch. f. Protistenk.* I., 3, 1902.

composition of *Paramœcium* wears out or disappears so that the ferment-forming activity is lessened. A change of diet restores the power. The restoration may be effected, not only by the extract of beef, but by clear chemicals as well. Thus potassium phosphate, potassium chloride, sodium and magnesium chlorides, have all given positive results in this direction, and it is probable that these substances contain the elements needed by the weakened protoplasm. We are justified, therefore, in regarding the artificial rejuvenation of *Paramœcium* as a phenomenon of the same order as the chemical fertilization of the egg.[1]

The general problem may be attacked from another point of view. What is it that conjugation does to renew the potential of vitality? The early conception of Butschli's that conjugation brings about a renewal of youth or a *Verjungung* of the participating organisms has been repeatedly confirmed by subsequent observers. A very significant fact came to light in the course of these experiments and one which seems to show that conjugation, like the action of the beef-extract, involves certain chemical phenomena. It will be recalled that Maupas maintained that endogamous conjugation is fruitless and the reason given was that, as the cultures grow older, the micronucleus degenerates and disappears. Maupas did not keep *Paramœcium* in culture for more than a few weeks, but the period was long enough for him to feel justified in assuming that this form is similar to the other forms examined by him. He declared that endogamous conjugations in this species (and he obtained many of them) are sterile because the micronucleus is lost through degeneration. I have preparations of this form made at intervals throughout the 665 generations, during endogamous and exogamous conjugation, at periods of depression and of vigor, and in none of them is the micronucleus absent. Upon this point Maupas was most assuredly mistaken.

From time to time As and Bs have been put together for the purpose of obtaining fertile conjugations. In the material set aside as "stock" conjugations have also frequently occurred, these of course, being endogamous. A comparison of "wild"

[1] Cf. Calkins. Studies on the Life-Cycle of Protozoa. I., The Life-Cycle of *Paramœcium caudatum*. *Arch. f. Entwickl.*, 1902.

conjugations with endogamous and exogamous unions is very instructive. By "wild" is meant pairs that are captured while conjugating in the natural pond water. There were only three pairs of these but of the six ex-conjugants five continued to live in the regular culture medium. Had the original number of pairs been larger, the result would be more satisfactory but I believe the proportion of fertile results would be as high as five out of six, or, expressed in percentages, as high as 83 per cent. Of the exogamous conjugations, *i.e.*, those of diverse ancestry, there were 24 pairs selected at different periods, and 48 ex-conjugants isolated, of which only three continued to live in the same culture medium, or about six per cent. Of the endogamous unions, *i.e.*, conjugations in which the gametes were of the same ancestry, 16 pairs in all were selected and 32 ex-conjugants isolated, of which two only continued to live, again about six per cent. How are we to account for the great discrepancy between the proportion of fertile wild ex-conjugants and those obtained from the cultures? *I believe that it can be accounted for on the ground of chemical differences in the composition of the protoplasm of the different individuals.* In the natural habitat of these forms no two individuals have the same environment for long periods and it may well be that no two parts of the pond have an identical composition. When two organisms unite, as *Paramæcium* unites, and part of the micronucleus of one unites with a similarly-derived part of the micronucleus of the other, the result is the formation of a new chemical substance in each organism, for the fusion nucleus in all probability is different in minute composition from either of the old ones. Now, on the other hand, when the organisms had been in the same culture medium for long periods, as was the case with the As and Bs of these experiments, there was no chance for them to be different from one another in chemical composition, and the ex-conjugants, after a few generations, died in the great majority of cases. In all such cases the conjugations were normal, micronuclei were exchanged, the old macronuclei became disintegrated and new ones were properly formed, but something was lacking and the ex-conjugants died. I believe this "something" has to do with the chemical make-up of nucleus and cytoplasm; and I further believe that the failure

which Maupas experienced in getting fertile endogamous ex-conjugants can be explained upon the same grounds. In one instance I treated an endogamous ex-conjugant with beef extract. The effect was most remarkable for it not only continued to live, *but it is still living in the 350th generation.* When it is remembered that the usual life-cycle of *Paramœcium* in culture is from 150 to 170 generations, this result is extremely interesting.

The facts of conjugation thus seem to fit in with the interpretation of the other results, to indicate that the protoplasm of this one-celled organism, like a galvanic battery, starts with a high potential of activity, which gradually fails with use, but which may be restored again and again by the proper chemical means. It is to such an assumption that we may turn for an explanation of the periods of depression that have occurred from time to time. The June period, at the 620th generation, appears to have been different from the earlier ones, for at this time, neither beef-extract, nor any of the salts that had been successful before, would restore the lagging energies. It was only the use of something entirely different from everything previously employed that finally saved the culture. It may have been the lecithine and allied substances in the brain extract, and it may have been the nucleinic acid in the pancreas extract; whatever it was, it apparently supplied something in the protoplasm of *Paramœcium* that had not given out before, but had failed at this particular time. Further, and more carefully-guarded experiments, must now be undertaken in the hope of finding out what these failures mean.

Turning again to the controversy regarding "old age" in Protozoa, it seems that we shall have to side with Weismann in the contention that these organisms have the potential of endless existence, and this without conjugation. The five times that the cultures of *Paramœcium* have been rescued at periods of depression, justify the belief that they may be saved as many times more, provided the time and patience required for such work are not out of all proportion to the results gained. It seems to be merely a matter of finding the right food, *i. e.*, the right chemicals. In nature, it is not improbable that the periods of depression, which are experienced in the laboratory, would be

absent, and that the division-rate would maintain a fairly uniform level. The chemical composition of a pond is constantly changing; every rainfall washes new salts into the water; every wind assists in turning the water over, and with these changes, it is probable that the organisms get new supplies of the substances necessary for their continued activities. When other things fail, the organisms may resort to conjugation, but we may consistently doubt whether this method of recuperation for Protozoa is as widespread in nature as hitherto supposed.

One other analogy is suggested by these experiments, *viz.*, the similarity of these periods of depression, and the subsequent proliferation, to tumorous growths of various kinds in the higher animals. The race of *Paramœcium* may be compared with the tissues composing the body of a Metazoön. When the cells composing these tissues approach the age-limit of the race, they divide less and less often; finally they come to a halt, so far as division is concerned, and senile degeneration begins, which ends in death. If, in a given tissue, after it has passed the period of active division, some stimulant be given at a local area, thus inducing a renewal of the dividing energy and proliferation of the cells of that area, the result, from a pathological point of view, would be a tumor; from a biological point of view, it might well be the rejuvenescence of the cells, as in the case of *Paramœcium*. As in the latter case, a number of agents might be responsible for calling out the increased activity, as, for example, a blow, a parasite, an enzyme, a changed condition of the immediate environment, etc. Any one of these might "rejuvenate" the tissue-cells in the same way that a chemical induces a new cycle of generations of *Paramœcium*.

Marine Biological Laboratory,
Woods Holl, August 6, 1902.

FURTHER NOTES ON THE COCOONS OF ALLOLOBOPHORA FŒTIDA.

KATHARINE FOOT AND ELLA CHURCH STROBELL,
Woods Holl, Mass.

The fact that cocoons of *Allolobophora* are formed during copulation challenges the interpretation that spermatozoa are stored in the spermathecæ in order to produce cross fertilization. We found, however, such conclusive evidence that the spermatozoa of the cocoon are derived from the spermathecæ, that this apparent inconsistency was stated in an earlier paper as a problem for further investigation.[1]

During the summer of 1901, we were able to make at least one step in this direction. We found that cocoons can be formed and deposited when the worms are not copulating. Isolated worms deposited cocoons for weeks, as many as ten cocoons being laid by one worm without copulation.

The method of experimenting was as follows: Single worms with a pronounced clitellum were isolated in half-pint bowls, each bowl being half filled with compost in which the worms had been found. Immediately after this compost was collected, it was carefully sifted to get rid of even the smallest worms, and it was then kept for three or more days before using. This was done to allow any cocoon already in the compost to reach a stage of development that would make it impossible to mistake it for a cocoon laid by the worm isolated for the experiment. At the end of three days the isolated worm was put in a bowl of fresh compost and the compost in which the worm had been kept was sifted under running water through a sieve with meshes too fine to allow a cocoon to pass. By this process nearly all the earth was washed away, and any cocoons present, were easily secured.

A perfectly fresh cocoon, *i. e.*, one with a slime-tube, was often found, but more often the cocoons contained eggs in a later

[1] The Cocoons and Eggs of *Allolobophora fœtida*, Jour. Morph., Vol. XIV., No. 3, 1898, pp. 496, 497.

stage of development, 2, 3, 4, and even 10 or 12 cells. When the stage of any egg appeared doubtful, it was fixed, stained and mounted for examination. Any cocoons in the compost before it was put in the bowls would have had a start in development of at least three days — so it was practically impossible to mistake them for the cocoons laid by the isolated worm.[1]

Twenty worms were isolated for the experiments described, and of these, one worm deposited ten cocoons at average intervals of three days. This worm was vivisected six days after depositing the tenth cocoon, and all four spermathecæ were found to be about one quarter full. The clitellum — which was very pronounced when the worm was isolated — had entirely disappeared, and the seminal vesicles appeared abnormal — they were a brownish-yellow, and quite different in appearance and texture from those of worms captured in the compost heap. This pathological condition, undoubtedly due to confinement, may have prevented the deposition of cocoons continuing until the contents of the spemathecæ was exhausted.

Of the remaining nineteen worms, one deposited four cocoons, and one deposited three, within fifteen days ; one worm deposited three in nineteen days ; two deposited two within seven days, and two deposited two within eight days. Three worms deposited each one cocoon on the third day, one worm deposited one on the fourth day, and one deposited one on the fifth day. In some of these cases the experiment was cut short by the escape of the worm.

Seven of the nineteen worms deposited no cocoon, vivisection showing, however, that two of them were pathological, and that the spermathecæ of four of them were almost empty. We found the spermathecæ full, and the seminal vesicles normal in only one of these worms, and there was no apparent cause why this worm should not deposit cocoons. These worms were isolated from thirteen to twenty days before vivisection.

The experiments described above establish the fact that a

[1] In order to become perfectly familiar with the different stages of development possible within three days, we preserved a large number of fresh cocoons, opening them at intervals of three hours, and fixing, staining and mounting the eggs.

cocoon can be deposited by one worm alone, and our earlier observation of the formation of cocoons during copulation, seems to prove conclusively that there are two methods of forming cocoons. These observations reconcile the opposing opinions[1] on this point.

That the deposition of cocoons by a single worm was not due to isolation, we determined by finding, in pots containing one

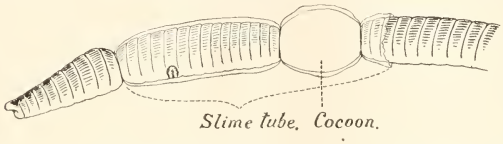


FIG. 1. × 3. Forty-two anterior segments of a worm with a cocoon in the process of formation. The transparent slime-tube surrounds the worm from the tenth to the thirty-sixth segment, and shows constrictions at each edge of the clitellum. The white cocoon encircles the clitellum. When the worm was dropped into boiling water, the cocoon became opaque — effacing the segments of the clitellum.

hundred worms, a number of single worms each with a cocoon around the clitellum. During the summers of 1901 and 1902, we found sixteen of these worms, with cocoons in different stages of development, ranging from a cocoon containing only a trace of albumen and no eggs to one with the full amount of albumen of a mature cocoon, with 28 eggs. These immature cocoons, when removed from the worm, are surrounded by a single slime-tube, resembling one half the double tube found around a copulating pair. This single cocoon lies near the posterior end of the slime-tube (Fig. 3), maintaining the relation that exists between the slime-tube and cocoons in the case of copulating worms, where the two cocoons lie at opposite ends of the double tube. Examination with a lens shows the clitellum of the worm encased by the cocoon and part of the slime-tube, the longer part of the tube lying anterior to the clitellum (Fig. 1). When the worm is not disturbed it will withdraw backward, through the slime-tube and cocoon, finally pulling the head through the smaller end of the cocoon (Figs. 2 and 3). This accords with

[1] We have not been able to find any record other than ours of actual observation of the deposition of cocoons by copulating pairs, or of single worms with cocoons in process of formation.

our observation of copulating pairs, for in these cases also, the longer part of the slime-tube lies anterior to the clitellum, each worm finally withdrawing backward through the small end of the cocoons. In the worm shown in Fig. 1, the slime-tube extends to the tenth segment, but it is not safe to assume that in the living worm the tube may not extend still further, possibly covering the spermathecal openings between the ninth and tenth, and tenth and eleventh segments. The sudden

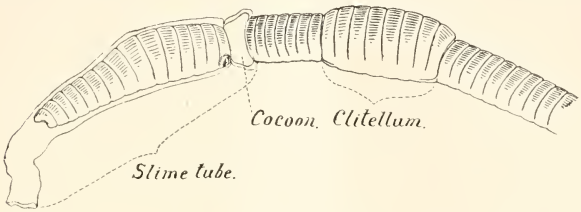


FIG. 2. × 3. Forty-two anterior segments of a worm caught with a nearly mature cocoon around its clitellum. Before being dropped into boiling water, the worm partly withdrew from the cocoon, leaving it encircling the 16th–18th segments. The cocoon and segments are very much contracted, the cocoon appearing as a narrow, opaque band around the worm. Such cocoons regain the typical oblong shape when removed from the worm. The worm has partly withdrawn from the slime-tube, leaving the anterior end projecting beyond its head.

extension of the worm, when dropped into boiling water, possibly causes such change in the position of the slime-tube, as shown in Fig. 1.

On the other hand, we have no proof that the contractions of the worm when captured do not result in an extension of the tube beyond the point covered under normal conditions, and that in the living worm the tube does not reach the spermathecal segments. In living worms, we have seen the slime-tube, in some cases, stop short of these segments, in others, completely cover them, but it is impossible to affirm which condition prevails when the worm is at rest. Fig. 3 shows a tube with its posterior limit clearly indicated, by the impress of five segments posterior to the clitellum. A comparison with Fig. 1 shows that the slime-tube in the captured worm stops short of this point.

Our method of securing cocoons in the process of formation

on single worms, was to dump daily several pots, each containing one hundred worms and carefully to examine the clitellum of each worm. The presence of a slime-tube, with or without a cocoon, can be detected, by constrictions at each end of the clitellum. These constrictions are very marked when the worm is contracted and difficult to see when the worm stretches out. When the worm succeeds in partially escaping from the slime-tube, one or both constrictions can be seen on, or near, the clitellum. These constrictions are produced by the slime-tube and

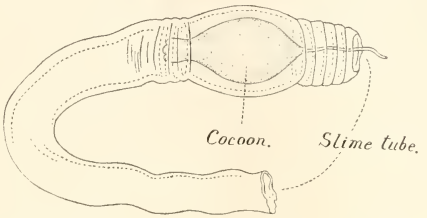


FIG. 3. A freshly deposited cocoon. When on the worm the shorter end of the slime-tube is posterior to the clitellum and the impress of 5 segments on the tube shows that, in this case, the posterior end of the slime-tube extented to the 39th segment of the worm. This figure was reduced one fourth from Fig. 3 of an earlier paper. The original figure was magnified seven diameters.

not by the cocoon, for we have found worms showing marked constrictions, when careful examination of the slime-tube after removal, failed to show any trace of a cocoon. This indicates that the formation of the slime-tube is the first step in the process of forming a cocoon.

Having established the fact that cocoons are formed by these worms, during copulation or separately, the opposing opinions on this point are harmonized. The question now remains, which method predominates?

We find eggs in the cocoon while it still encircles the clitellum, proving that they must be conveyed through the slime-tube, from the oviducts on the 14th segment, to the cocoon; and that they are not deposited in the cocoon when it finally passes over these segments, as suggested by several investigators.

We have found from one to twenty-eight eggs generally near the anterior end of the cocoon. In some cases these cocoons

were so little advanced in development that without a lens they could not be detected until the slime-tube was removed from the worm. Examination of the tube revealed an immature cocoon, with a white semi-transparent covering, containing a much smaller quantity of albumen than is found in mature cocoons, but the eggs showed the stage of development found in a freshly deposited cocoon, *i. e.*, the first maturation spindle at the metaphase and in the center of the egg. Evidently the delicate covering of the cocoon is formed before the albumen is deposited, but the substance that forms the chiton of the covering of the older cocoons is not present, for we have preserved these immature coverings in moist earth for twenty-four hours, and they failed to become yellow and tough, like the covering of the mature cocoon under these conditions; they were white and soft at the end of the experiment.[1] When the slime-tube is torn from these immature cocoons, we find the covering of the cocoon consists of two layers; an outer, perfectly transparent layer, that can be slipped off the inner covering, which is thinner, more resistant, and semi-transparent. The former appears to be part of the slime-tube substance that adheres to the cocoon, but in later stages, when the cocoon is ready for deposition, this layer if preserved becomes yellow like the under layer, which indicates it to be part of the covering of the mature cocoon.

Spermathecæ.

In 10 per cent. of a large number of worms dissected, each with a pronounced clitellum, all four spermathecæ were found empty, this proving that the appearance of the clitellum does not indicate whether the spermathecæ are full or empty, and also that cocoons can be formed until the spermathecæ are entirely empty. In a few cases we found the spermathecæ of copulating worms empty, even when several spermatophores were present in the slime-tube over the spermathecal segments, showing that, spermatozoa are not received into the spermathecæ during the early stages of copulation.

The condition of the individual spermathecæ varies greatly. Sometimes we found only one of the four entirely empty, and

[1] In only one case, an immature cocoon, with a trace of albumen and one egg, showed this characteristic yellow tinge after three hours in water.

again only one full, while the contents of the other three varied in amount. We were unable to discover any constancy in these relations.

Spermatophores.

Having often observed the deposition of cocoons by copulating worms, one of us[1] was led to conclude that the spermatophores in the double slime-tube represented the first step in the formation of the two cocoons. If this were true, we should find spermatozoa in immature cocoons at the earliest stages. The albumen from the immature cocoons found around the clitellum of single worms was dried on slides, stained with iron hæmatoxylin and examined under a Zeiss 4-mm. and a 2-mm. immersion lens, with the aid of a mechanical stage. This albumen resembled that found in freshly deposited cocoons, but *did not contain* spermatozoa. It is evident from the above, that the spermatozoa are put into the cocoon at a later period, possibly when the cocoon passes over the spermathecal openings as it is deposited. The fact, however, that the slime-tube shown in Fig. 1 extends to the posterior spermathecal openings suggests that in its normal position the tube *may* extend to enclose all four spermathecal openings, and the spermatozoa like the eggs be conveyed through the tube to the cocoon, before deposition. But the albumen of three cocoons apparently just ready for deposition failed to show any spermatozoa. These cocoons tightly encircled the clitellum and were equal in size to a freshly deposited cocoon; one contained eleven eggs, two normal; one contained twelve eggs, six normal; and one contained twenty-eight eggs, five normal. The white covering of one cocoon after one hour; of one after twenty minutes; and of one after ten minutes, in water, became tough and yellow like the fresh cocoon a few minutes after deposition.

That spermatozoa are not found in nearly mature cocoons, and that spermatophores *are* found in the slime-tube of copulating worms before the covering of a cocoon is formed, proves beyond question that the spermatophore is not a device for conveying spermatozoa into the cocoon. It would seem rather to be a device for filling the spermathecæ, and it is not formed *after* the spermatozoa leave the spermathecæ, as conjectured in an earlier paper.[2]

[1] *Loc. cit.*

[2] *Loc. cit.*, p. 498.

Spermatophores, however, are certainly not received into the spermathecæ in the form of spermatophores, for we have vivisected many copulating worms having spermatophores at each end of the slime-tube (*i. e.*, over the spermathecal openings of both worms) and the contents of the spermathecæ, in every case, was quite unlike the spermatophore substance; the former mixing freely with water, while the spermatophores do not disintegrate even after twenty-four hours in water.

The granular substance secreted by the spermathecal glands[1] which forms the center of the spermatophores also fails to disintegrate in water, and we have not been able to identify this substance in the spermathecæ.

When copulating worms are allowed to withdraw from the double slime-tube, we sometimes find aggregations of spermatophores in the definite areas of the slime-tube where the two cocoons are formed. The constrictions of the slime-tube that close each end of the cocoons, confine the spermatophores to these areas, and when filled with spermatophores, these areas bear such a striking resemblance in form and color to freshly deposited cocoons, that in our earlier observations we misinterpreted them as partly formed, or nearly completed cocoons. The true "partly formed cocoon" has a white semi-transparent covering, contains more or less albumen and several eggs. Prior to 1901 we had found these cocoons only in the compost not around the worm. We found only a few, and in every case the cocoon contained eggs, but as we did not preserve the albumen, the absence of spermatozoa was not discovered and we supposed these cocoons to represent a later stage of development than the aggregations of spermatophores in the slime-tube.

The marked difference in structure of the spermatophore substance and the contents of these cocoons was noted, as well as the fact that in freshly deposited cocoons we found only a very small number of spermatozoa, in comparison with the large quantity found in a slime tube containing spermatophores. It was these inconsistencies that prompted us to undertake the experiments described above.

[1] *Ibid.*, p. 496.

A BIOLOGICAL FARM.[1]

For the Experimental Investigation of Heredity, Variation and Evolution and for the Study of Life-Histories, Habits, Instincts and Intelligence.

C. O. WHITMAN.

The biological laboratories of to-day, in design, equipment and staff, are almost exclusively limited to the study of *dead* material. Living organisms may find a place in small aquaria or vivaria, but they are reserved, as a rule, not for study, but for fresh supplies of dead material. It is no disparagement of the laboratory to point out a broad limitation in its ordinary functions and the pressing need of new facilities for observation and experiment on *living* organisms.

The fundamental problems of heredity, variation, adaptation and evolution cannot be wholly settled in the laboratory. They concern vital processes known only in living organisms—processes which are slow and cumulative in effects, expressing themselves in development, growth, life-histories, species, habits, instincts, intelligence. These problems require, therefore, to be taken to the field, the pond, the sea, the island, where the forms selected for study can be kept under natural conditions, and where the work can be continued from year to year without interruption. Such a field, combining land and water, and stocked with animals and plants, and provided with a staff of naturalists, would have the essentials of a biological farm, now justly considered to be one of the great desiderata of biology.

This great need (pointed out in all our annual programmes since 1892, and named as one of the three leading purposes of the Culver endowment) has been felt ever since Darwin's time, and has been strongly urged by such evolutionists as Romanes, Varigny, Galton, Weismann and Meldola. Thus far the project has not been realized, except on a small scale through individual effort.

[1] Read to the Corporation of the Marine Biological Laboratory, at the annual meeting, August 12, 1902.

The most notable move in this direction is that of Professor Cossar Ewart, of the University of Edinburgh. "The Penycuik Experiments"—the first product of Professor Ewart's enterprise—form a brilliant illustration of the kind of fruit to be expected from a farm devoted to experimental research. Single-handed, Professor Ewart attacks the problems of heredity, and quickly shows how decisive are direct experiments in dealing with such subjects as telegony, prepotency, reversion, inbreeding, etc.

The plans proposed by Romanes and Varigny had as chief ends in view demonstrative tests of the theory of the origin of species by natural selection. But the contest between the old belief in the immutability of species and the new doctrine of descent has been decided, and the original idea of the farm has consequently ceased to have great influence.

The functions to be fulfilled by a farm are no longer prescribed by the exigencies of theories, but by the deeper and broader needs of pure research on *living* organisms. The problems of heredity and variability are fundamental, and naturally form the center of interest. Variability is the source of new species and the fountain of all progressive development in the organic world. In heredity lies the power of propagation and continuity of species. These are inexhaustible subjects, from the investigation of which must flow rich accessions to knowledge, which will redound to the advancement of human welfare.

These subjects are in some aspects and details amenable to laboratory research; but for the most part they can only be effectively dealt with under conditions represented in the farm. This holds, for example, in that most promising branch of experimental biology—*hybridisation*. Botanical gardens and zoölogical parks have been utilized to some extent in this work, but they are adapted to show-purposes, and of little value for research of this kind. The far-reaching importance of this subject, both for science and practical breeding purposes, is well attested in Mr. Ewart's experiments, in those of Hugo de Vries, as recorded in his monographs on the origin of species in the plant world, and again in Mr. Bateson's "Experimental Studies in the Physiology of Heredity."

The functions of a biological farm are not all summed up in experimentation. That old and true method of natural history—*observation*—must ever have a large share in the study of living things. Observation, experiment and reflection are three in one. Together they are omnipotent; disjoined they become impotent fetiches. The biology of to-day, as we are beginning to realize, has not too much laboratory, but too little of living nature. The farm will certainly do much to mend this great deficiency. The farm would enable us to work out life-histories, bring us face to face with instinct, put it under control, so that we could handle it, photograph it, analyze it, read its history, and extort from it an answer to the question, Whence and how came intelligence?

It would enable us to extend the study of development beyond the stages represented in the egg and the embryo to those leading up to mature age, and thus bring within reach vast series of most important data for the study of evolution.

In such data we might expect to see to what extent individual development recapitulates race development, and to get important clues to the meaning of this so-called biogenetic law. The whole meaning of development and heredity is involved in these phenomena of "recapitulation." That the first step in recapitulation is the germ-cell, we know. The fertilized germ, or egg, passes through a series of form-stages, leading through the morula, blastula, gastrula, embryo, larva, etc. Whether these stages epitomize the ancestral series is a question very difficult to decide, and opinion is much divided. This obscure but fundamental problem of development can probably never be solved by embryological data alone. Paleontology throws much light on the general question, but deals entirely with non-living remains. If there be recapitulation, it should certainly be discoverable in post-embryonic stages, where characteristic features are slowly elaborated and brought to a completion in detail quite beyond the possibilities in earlier life. Strange to say, these later stages have been but little studied in living forms, museum morgues having been the chief reliance. It is in these stages that recapitulation may be actually seen as a life-process, successive steps in evolution repeating themselves with sufficient fullness to satisfy the most skeptical. Such sequences are often manifest in the

development of instinctive behavior, and even in voice-changes and food-instincts at certain life-epochs corresponding seemingly to evolution-epochs. Remembering that the distant ancestors of land animals were undoubtedly aquatic, the history of individual development in amphibious forms of to-day becomes intelligible as an abbreviated and variously modified record of race development. Making all allowance for secondary adaptive changes, it is nevertheless safe to say that race evolution is sketched in the development of the individual—sketched not only in fundamental features of structure, but also in the accompanying physiological and psychological changes.

Reminiscences of aquatic life are seen not only in land animals that return to the water to deposit their eggs, but also in all the higher animals, since they begin life in the unicellular stage and require for their first development to be bathed in fluid.

Sequence in color-patterns, so characteristic of young animals of almost all species, and especially so of birds, furnishes innumerable illustrations of the biogenetic law, and in many cases, where only two extremes of the sequence are present, it is possible by simple experiment to bridge the gap, and thus to show that the two extremes are really two stages of a continuous development. For example, in some wild species of pigeons we find that the color-pattern of the first plumage succeeding the down is so different from that of the second (adult) plumage, as to appear to have no direct developmental relation to it. By plucking one or more feathers from the first plumage at differen times before the first moult, intermediate stages can be obtained, showing precisely how the first pattern can be progressively converted into the second. Such experiments enable us to force from nature more complete records of her past and present doings.

Work on living organisms, dealing with such subjects as heredity, variation, adaptation, correlation, development, recapitulation, hybridisation, origin of species, nature of specific characters, life-histories, habits, instincts, intelligence, etc., requiring uninterrupted continuance from year to year for long periods, under conditions that secure most favorable control for experimentation and study, calls for facilities which have yet to be provided.

There is no quite satisfactory name for the new plant required for such work, and no one has suggested a practical method of developing it. Biological Farm, broadly defined, is perhaps the best we can do for a name, as the work would be, so far as possible, upon plants and animals under cultivation. A considerable tract of land, of varied surface, including woods, fresh-water ponds, some brackish water, and a stretch of sea-shore that could be utilized in the cultivation and study of marine animals, would represent the essentials of the farm headquarters.

The best location for the farm would be in the immediate vicinity of a laboratory holding the position of a national center of biological research. The Marine Biological Laboratory has a good prospect of becoming such a center, if it is not one already, and its proximity to the United States Fish Commission Station only insures additional advantages, of great importance to the farm. The farm, the laboratory, and the station would be reciprocally helpful and stimulating, and in many problems the three could work hand in hand, each supplementing the work of the others. These three establishments would form a most comprehensive biological center, such as has never been equalled.

In dealing with the problems of heredity and variation, it is of the highest importance to know the *history of the material* to be investigated. It is this prime essential that is so conspicuously missing in most of the work hitherto done in these lines. Curves and formulæ may be all right mathematically and yet all wrong biologically. Even Galton, the father of the statistical school, warns us that "no pursuit runs between so many pitfalls and unseen traps as that of statistics." (Biometrika, I., p. 8.)

The farm will furnish material with *exact records*, and will thus render a most important service to laboratory workers. A single illustration will suffice. It has been discovered that the paternal and maternal chromosomes in the cross-fertilized egg remain distinct, at least in the earlier stages of development. This seems to account for the fact that hybrids of the first generation between distinct species are generally "intermediates." When these hybrids breed *inter se* or with the parent species, we often get so-called "reversions." Hitherto we have not found any explanation for these "reversions." The solution of this

most interesting problem in heredity only waits for the right material with precisely defined origin, and for this the laboratory could look to the farm. But the farm would do more than supply the needed material; its records and experiments would suggest the theory and give the physiological test, while the laboratory work would find the morphological test. The coöperation between laboratory and farm would thus be intimate and of inestimable value in a multitude of ways.

It must be remembered, moreover, that Wood's Holl has great natural advantages as a location for the farm. Ever since Baird's time it has been generally known that this is the best place on the Atlantic coast for the study of marine biology. Indeed, Wood's Holl has become a strong biological center by virtue of the exceptional opportunities for work here offered in a varied and extensive shore fauna and flora, in numerous accessible islands rich in forms of peculiar interest, and in many perfectly isolated fresh-water ponds, brackish ponds, and salt-water ponds of easy control. These attractive features, together with a climate suited to both winter and summer work, certainly comprise the essentials of a good location.

A further consideration in favor of this location is the fact that here for the first time in this country the farm project was definitely formulated; and, what is more to the point, it is here that the first step in the development of a farm has been taken, and the work carried forward for some six years by individual endeavor. The birthplace of an enterprise is not likely to be a pure accident. In the present case it was certainly determined by the various causes which have conspired to make Wood's Holl a biological center. This center has at least fifteen years of growth, and every year makes it stronger and increases its importance as a location for the headquarters of a biological farm.

The argument for location has already suggested that the farm is not necessarily limited to a single tract of land. It is designed to supplement, and coöperate with, a laboratory, and hence must have its headquarters conveniently near. There is no reason, however, for limiting its territory to the ground selected for this purpose. If, to take a familiar example, a study of the terns were to be undertaken, we should undoubtedly resort to Peni-

kese, taking advantage of the headquarters selected by the birds. If we were to take up some groups of migratory birds, we might find it desirable to migrate with them, changing our location to suit their summer and winter predilections. For forms settled in tropical regions, longer excursions might be necessary, and this might lead to the development of a new farm. These possibilities do not in the least conflict with the plan proposed for Wood's Holl. No matter where a farm be located, it must have headquarters, and occasionally extend its field of work to more or less distant points of interest.

The headquarters must be in close touch with a laboratory, and both should be in a place where the natural advantages and the organization are such as to draw a large number of investigators — most emphatically not in a place that invites a large number of spectators, as in the public parks of cities. In this latter respect Wood's Holl is most perfectly adapted to the purpose, and the prospect is good that we shall never be troubled with throngs of summer visitors. This is an ideal feature in the situation that it would be hard to duplicate.

The practical question arises as to how to proceed with the development of a farm. Our limited experience strongly confirms the opinion with which we set out, namely, that the best method is to develop the farm slowly, section by section. Each section should be a group of related species, selected with a view to combining a wide range of problems. It should be developed and directed by an investigator prepared to make it his life-work. This investigator, or director, should have the support of a number of assistants competent to deal with special problems, one or two artists, a photographer, a stenographer, a keeper and a business superintendent.

Developed in this way, the cost of maintenance would not be heavy at first. Ten thousand dollars a year would support a large and thriving section. The multiplication of sections and the gradual growth of the work would call for a larger income. A farm of ten large sections would require an endowment of a million, and it is easy to see room for many millions.

If the scheme here outlined approaches the ideal which science is waiting to see realized, it will be seen that the farm does not

find its chief purpose in demonstrations of the truth of evolution or in testing the theory of natural selection. It is not a project designed simply to turn out curves and formulæ for the delectation of the knight-errants of statistical lore, nor is it the particular pet of any school or fad. Moreover, the prevailing idea that it has something in common with a zoölogical or botanical park rests on a total misapprehension. The organization, management and all the conditions obtaining in the public park are incongruous with those required for a research farm. Heterogeneous collections of animals, exhibited for the amusement of people, are wholly unsuited to the purposes of investigation in time, place and character. For the kind of work contemplated, the investigator must have forms of his own selection, collected, arranged and kept for his special purposes. He must have complete and permanent control of his quarters and the forms he is to study, and above all, *complete isolation from the public*. Only under such conditions could he have the unbroken quiet required in delicate observation, or expect natural behavior from the forms occupying his attention.

The farm project, let me say in conclusion, is one we cannot afford to see drawn away from Wood's Holl. It is an undertaking born and nurtured here, on a small scale to be sure, but still sufficient to give results and make clear the direct path to a large and most important development.

Hitherto we have felt perfectly secure in our right and ability to control the direction and scope of our development. Whatever compensation we may receive for the surrender of rights that go with the transfer of ownership to another institution, let us have no illusions. We shall henceforth only be a few among many advisors on the questions that concern the scope of our work and the enterprises that shall be permitted free development here. We shall undoubtedly be left quite free to manage the details of our work, but the decision of matters of far more fundamental concern to all who are deeply interested in the ideal development of a real *biological* center here — questions of the broader limits and character of our work — the decision of all this will certainly not be wholly with us. Not to realize this, would be woful blindness to the situation. The extent to which we can have and

hold our ideals will depend on the influence we can bring to bear, and in this all-important matter the Corporation can have, and will have, I trust, earnest work to perform.

Summary Statement.

Laboratories.—The biological laboratories of to-day are almost exclusively devoted to the study of "pickled" animals and plants. They have very few and inadequate facilities for the study of *living* organisms. This is a limitation for which no remedy has thus far been provided.

Field-work.—For the study of development, growth, life-histories, species, habits, instincts, intelligence, heredity, variation, adaptation, hybridisation, etc., we can neither depend upon the laboratory nor upon the field-work of naturalists. A world-wide field in which there can be no *control* of the forms to be studied, and no possibility of *continuity* in observation or opportunity for experiment obviously does not meet the requirements of science.

Biological Farm.—The laboratory is too narrow, and the world too wide for the continuous study of living organisms, under conditions that can be definitely known and controlled. For such study, selected groups of organisms and a limited territory, with favorable conditions of land, water and food are needed. Territory, living organisms and scientific staff would constitute a new plant, which might be called a *Biological Farm.*

Grounds.—The grounds of a biological farm would vary in extent with the growth of the work, from ten to a hundred or more acres. Land, woods, fresh-water ponds, sea-shore and islands would make a good combination.

Location.—The location of headquarters should be at a biological center, near laboratories drawing a large number of investigators, but at a safe distance from any large city or summer resort. These prime advantages of situation superadded to the exceptional natural advantages of available grounds are represented at Wood's Holl.

Natural Conditions.—A biological farm should include the largest possible diversity of natural conditions to insure wide freedom of development and opportunity for experimentation.

Water is the first essential, and three forms of this element are needed, namely, sea water, brackish water, and fresh water. All three forms abound at Wood's Holl and vicinity. The fresh-water ponds are numerous, and many of them, both on the main-land and on the neighboring islands, are *completely isolated* and stocked with forms in-bred for centuries. Brackish water in almost every degree, pure sea-water and tide-currents are right at hand.

Land is the next essential, and here we have hill, plain, marsh, swamp, shore and islands, and some of these islands are inimitable biological farms of nature's own make.

A seasonal range of temperature is essential to the existence of a majority of the forms best suited to cultivation and study; and in this are supplied very important conditions for experimental work. The surrounding sea protects Wood's Holl from the extremes of heat and cold.

Isolation.—The most favorable combination of conditions may be utterly worthless, unless the farm can be made secure in its isolation from the public. Its work must go on in a *quiet environment*, where all the conditions are under control, and the investigator is free from the danger of intrusion.

Mode of Development.—The work should be developed slowly, section by section, each section consisting of a group of related species, or a single species, offering a wide range of problems.

Each section should be in charge of a director, prepared to continue the work during life, and supported by assistants and help for all routine and mechanical service. The staff would consist of directors, assistant investigators, artists, photographer, clerical help, keepers and a business manager.

Outlay and Maintenance.—The original outlay for land, stock, buildings, equipment, inclosures of land and water for isolation purposes, would vary according to the forms selected for study. From $50,000 to $100,000 would suffice for this. The maintenance of the first section, including salaries, accessions to stock, library, etc., may be estimated at $10,000 a year. The cost of additional sections would be about $5,000 each.

Ideal Center.—The association of three such institutions as the Marine Biological Laboratory, the U. S. Fish Commission Sta-

tion, and a Biological Farm would form an ideal biological center. Each would help and be helped by the other two.

Coöperation.—There should undoubtedly be several biological farms in the country. The larger universities might well have their own farms, and thus very extensive and effective coöperative work be carried on.

Use to Science.—The farm would enable us to approach all the fundamental problems of life from the two sides of observation and experiment on living organisms. It would furnish material for study with precise records, and make it possible to keep up continuity in the experimental study of heredity and variation.

Practical Utility.—The utility of such work is seen when we reflect on the practical results already realized in the multiplication and improvement of domestic species of animals and plants through cross-breeding, hybridisation and selection. We have very meager and uncertain knowledge of the laws of heredity and variation—laws which underlie all progress of the race.

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. X. THE MOVEMENTS AND REACTIONS OF PIECES OF CILIATE INFUSORIA.[1]

H. S. JENNINGS AND CLARA JAMIESON.

The present paper records an attempt to investigate by operative procedure the division of labor among the organs of locomotion of the ciliate body; to determine the part played by cilia of different position or structure in the usual movements and reactions. In many of the Ciliata the body is much differentiated, often bearing a number of different sorts of simple or compound cilia, variously distinguished as membranellæ, cirri, setæ, cilia proper, and the like. In such organisms as *Stylonychia* (Fig. 1) and *Stentor* these different structures have a characteristic distribution, and apparently differ markedly in function. Even in less differentiated infusoria a distinction between oral or peristomal cilia and body cilia can usually be made.

Observation of the behavior of these animals indicates that there is a division of labor among these cilia in the production of the usual movements. The organisms as they swim through the water follow a spiral course, usually swerving continually toward one side, but compensating the deviation thus caused by rotating on the long axis. The swerving is as a rule (though not invariably) toward the side away from the peristome or the oral cilia. The stroke of the oral cilia is of such a character as to tend to turn the body toward the side opposite to that on which they are situated, so that the swerving appears to be due to these cilia,—aided, as a rule, by the form of the body.

In reacting to most stimuli, these organisms turn toward a structurally defined side (see Jennings, 1900), and, as a rule, this is again the aboral side, or that opposite the oral or peristomal cilia. Observation indicates that this is due to the stroke of the oral cilia, or in some cases to a still smaller and more localized group of cilia. This has been set forth by a number of authors,—

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 56.

notably by Pearl (1900), in the case of *Colpidium*, and by Pütter (1900, p. 273), in *Stylonychia*. In *Colpidium*, according to the observations of Pearl, it is a group of cilia at the anterior end of the body which, invariably striking toward the oral side when the animal is stimulated by the electric current, cause the body to turn toward the aboral side. In *Stylonychia* it is the entire group of peristomal cilia that, striking toward the oral side, cause the body to turn toward the aboral side.[1] We can testify from our own observations that these relations seem clear. One naturally concludes, therefore, that the sidewise motion is determined by these peristomal or anterior cilia, and that if they were removed the motion would no longer occur. Massart (1901, p. 27), on the basis of Pearl's work, accepts the idea that the sidewise movement is caused by different cilia from the backward or forward motion,—aiding thus to distinguish between what Massart calls *phobism* (the backward movement in reaction) and *clinism* (the lateral movement).

The work here set forth was undertaken for the purpose of getting further light on this and related matters. If the anterior or oral cilia are removed by operation, will the organism no longer turn toward the aboral side when stimulated? And is the character of the spiral swimming modified by removal of such cilia? In general, does the removal of specific groups of cilia (with the accompanying alteration of the body form) have specific effects on the movements of the animal?

The behavior of pieces of infusoria has been studied by Verworn (1889) and Balbiani (1888).[2] The results of these investigators are of the greatest interest, but do not touch specifically the questions raised above. They found that pieces of ciliates move in general in the same manner as do the entire organisms. The reaction to stimuli by turning toward a certain structurally defined side, and the swerving toward a structurally defined side in the spiral course, in the uninjured animal, had not at that time been observed, so that these matters were, of course, not observed for the pieces. The questions proposed in this paper were therefore not touched by the experiments of Verworn and Balbiani.

[1] These are aided in the turning, according to Pütter, by the "running cilia."

[2] Balbiani's paper we have been unable to consult, so that we must depend upon the account of it given by Verworn.

In giving an account of the results of our experimentation, matters which were described by Verworn (1889) will not be dwelt upon, but the account will be confined to the points bearing on the questions raised above.

The operation of cutting the animals was done with a small knife, under the Braus-Drüner stereoscopic binocular, which has great advantages for such work. It was found best to cut the animals when resting against a clean glass surface. Most of the operations can be performed by the aid of some patience without great difficulty. In all cases the results set forth are drawn from experimentation and observation on a considerable number of specimens.

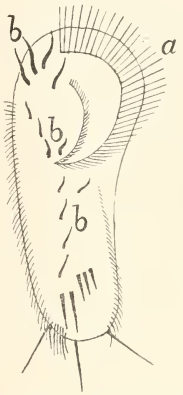


FIG. 1. *Stylonychia*, ventral surface, showing the distribution of locomotor organs (after Pütter, 1900).

1. *Stylonychia*.—Pütter (1900) gives a full account of the normal movements of this animal when uninjured, with a careful analysis of the action of the different cilia in producing the movements. For our purposes it is important to note the following different movements. (1) The animal runs forward on surfaces, chiefly or entirely by the aid of the "running cilia" *b*, Fig. 1. (2) When stimulated it jerks back a little, then turns to the right or aboral side. This turning appears to be due to the lateral stroke of the large peristomal cilia *a*. (3) The animal may also swim freely through the water; in doing so it swerves continually towards its right side, and revolves on its long axis from right over to left—the resulting path being a spiral. The swerving toward the right appears to be due to the lateral stroke of the peristomal cilia, and the revolution on the long axis might well be due to the same factor.

When *Stylonychia* was cut in two transversely just behind the mouth, so that the entire peristome remains with the anterior half, the pieces move as follows:

1. Anterior half, possessing peristome (Fig. 2, *a*).—After the first shock effects, the movement throughout is nearly like that

of the uninjured animal, but somewhat more rapid than usual. The piece runs forward on the bottom, turning to the right when stimulated. It differs from the entire animal in that it much more rarely jerks backward when stimulated, though it does this when the stimulus is powerful. When swimming through the water, it revolves to the right and swerves to the left, as in the entire animal. Since the anterior half possesses the peristome, the similarity of its movements to those of the uninjured animal was to be anticipated.

FIG. 2. Anterior (*a*) and posterior (*b*) halves of *Stylonychia*.

2. Posterior half, without peristome (Fig. 2, *b*).—Immediately after the operation the piece jerks rapidly backward, turning at the same time toward the right side. After a time it becomes quieter, and now it reacts essentially like the entire specimen, though it is somewhat less active. If stimulated with the glass hair at the posterior end its runs forward; stimulated at the anterior (cut) surface, it jerks backward and turns to the right. Chemical stimuli cause the same reactions. A little m/20 NaCl, or a weak solution of methylene blue was introduced with a capillary tube close to the piece; on coming in contact with the chemical the piece backs and turns to the right, just as is done by the entire animal.

Thus the presence of the peristomal cilia is not the determining factor for the invariable turning to the right as a response to stimuli.

3. Posterior one fifth. — The movements are somewhat less well coördinated than in the larger pieces. But after the shock effects have ceased, the piece creeps forward, *turning to the right*. It swims slowly through the water, swerving to the right and revolving to the left, as in the uninjured specimen.

4. Anterior one third. — This is very rapid in its movements, and circles almost continuously to the right. In the free water it revolves in the usual way. When stimulated, it turns to the right, but *does not jerk back*.

5. Middle one half. — This was obtained by cutting off the anterior and posterior fourths of the body. Its movements and reactions are essentially similar to those of the uninjured specimen.

6. Right and left halves. — These were obtained by splitting *Stylonychia* lengthwise. They move and react (after the shock effects have ceased) in nearly the normal manner. Thus, when stimulated mechanically or chemically, they jerk backward, and the right half turns toward its right (uninjured) side, while the left half likewise turns toward its right (injured) side.

A specimen was cut lengthwise in such a way that the left piece was a strip comprising about one third the body. This moved somewhat irregularly, but reacted in the same manner as the left one half just described — though it evidently bore no part of the peristome.

7. A small, left posterior corner, comprising less than one fourth the animal, reacted, after the effects of the shock had ceased, in the usual way — by backing and turning toward the right.

Thus, on the whole, pieces of *Stylonychia* from any part of the body, if amounting in size to as much as one fourth to one half of the animal, move and react essentially like the entire specimen. In very small or very irregular pieces the movements become irregular, as might be expected.

Similar results were obtained with *Oxytricha fallax*, and with a number of unidentified *Hypotricha*, though the smaller size makes most other species less favorable for such experimentation than is *Stylonychia*.

II. *Stentor cæruleus*. — In *Stentor* the oral (or adoral) cilia or membranellæ form nearly a circle at the broad anterior end of the body. *Stentor*, when free swimming, follows a spiral path, revolving from right over to left. It reacts to chemical and mechanical stimuli by backing a little, then turning toward the right aboral side. It is evident to observation that the adoral cilia play a large part in the turning; from their large size and their posi-

tion at one end of the body it would be natural to conclude that the turning is due to them alone.

1. The entire anterior disk, with the adoral cilia and the mouth, was removed by a transverse cut near the anterior end (Fig. 3). The posterior portion (*b*, Fig. 3), having only the body cilia, swam through the water as usual, in a spiral, revolving from right over to left. The aboral side in *Stentor cœruleus* is usually curved in a very different manner from the oral side, so that it is possible to distinguish the two sides even when the disk is removed. The specimen deprived of the disk and anterior cilia reacted to mechanical and chemical stimuli by backing and turning toward the aboral side, just as does the uninjured specimen.

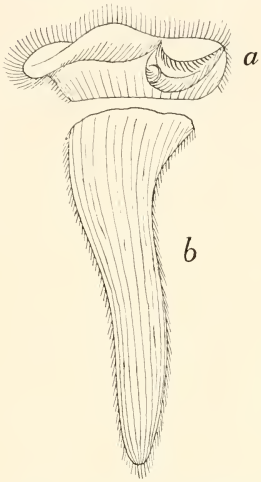


FIG. 3. *Stentor*, cut transversely just behind the disk.

After a time the specimen attached itself by the foot, and extended in the usual manner. Currents, due to the ciliary action, then passed backward toward the foot, or at times they were reversed, passing in the opposite direction. There was, however, no evident whirlpool formed, with a definite center, as in the uninjured specimen. The animal from which the disk had been removed, after becoming attached, reacted to mechanical stimuli by turning into a new position, and later by contracting, just as happens in the uninjured specimen. (On the behavior of *Stentor*, see Jennings, 1902.)

2. The disk portion (Fig. 3, *a*), bearing the adoral cilia and the mouth, also moved and reacted in the usual manner—revolving to the left as it swam, and responding to stimuli by backing and turning toward the aboral side.

3. Posterior one fourth—comprising about one half the slender "stalk" of *Stentor*.—This swims about, revolving to the

left. Whether on stimulation it turns toward a definite side could not be determined. After a time the small piece fixed itself by the foot and extended. The currents caused by the cilia were most of the time directed away from the attached end, being thus the reverse of the normal currents. At times, however, the current passed toward the foot.

When stimulated mechanically, the piece responded, like the entire *Stentor*, by bending into a new position, and later by contracting.

III. *Spirostomum ambiguum.* — Essentially the same results were obtained with this large infusorian as with *Stylonychia* and *Stentor*. Both the anterior half, bearing the adoral cilia, and the posterior half, without adoral cilia, move and react in much the same way as does the uninjured individual. They revolve to the left when swimming freely through the water, and turn toward the aboral side when stimulated. The anterior half moves more rapidly than does the posterior half, and when stimulated turns through a much greater angle than does the latter. The posterior half, if not strongly stimulated, frequently remains nearly quiet, or oscillates back and forth, as described in the third of these studies (Jennings, 1899). If stimulated mechanically or chemically, however, it backs and turns toward the aboral side.

A posterior one third of the body reacts in the same manner as the posterior one half.

A minute piece from the posterior end, less than half the length of the contractile vacuole, moved in the normal fashion, revolving to the left, and moving with the anterior (cut) end in front. The reactions to stimuli in such a small piece are very difficult to determine.

IV. *Paramecium caudatum.* — *Paramecium* is, of course, much less favorable for work of this character than are the infusoria of which an account is given above. This is due in part to the minute size and rapid movements of *Paramecium;* in part to the strong tendency for the cut pieces to disintegrate at once. But, with much patience, satisfactory anterior and posterior halves can be obtained.

1. Anterior half, bearing the oral groove and mouth (Fig. 4, *a*). This moves and reacts almost exactly as does the entire animal,—

revolving to the left, and turning toward the aboral side when stimulated mechanically or chemically.

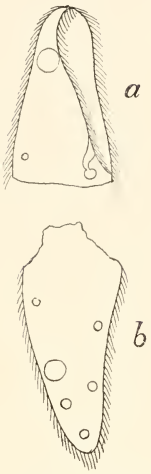


FIG. 4. Paramecium cut transversely. *a*, anterior half, with mouth and oral cilia. *b*, posterior half, without mouth or oral cilia.

2. Posterior half, without oral groove or mouth (Fig. 4, *b*). Much pains was taken to remove the entire oral groove and mouth, so that the posterior piece was usually a little less than one half the body. It was possible to distinguish the aboral side by the presence there of the contractile vacuole. The posterior half swims in a spiral, revolving, like the uninjured animal, to the left, and keeping the aboral side to the outside of the spiral. Its movements are slower than those of the anterior half, and it is much less sensitive, not reacting so readily to stimuli. The reaction to mechanical and chemical stimuli is by jerking backward and turning toward the aboral side, as in the uninjured individual. Thus the presence of the oral groove and oral cilia is not necessary in order that the usual movements and reactions may occur.

3. Middle third of *Paramecium* (anterior and posterior ends removed). — This behaves essentially like the anterior and posterior halves, or the entire animal.

SUMMARY AND CONCLUSIONS.

In all the Ciliata studied, including several of the Hypotricha (*Stylonychia*, *Oxytricha*, etc.), two of the Heterotricha (*Stentor* and *Spirostomum*), and one of the Holotricha (*Paramecium*), the movements and reactions of pieces of the organisms, if these are not too minute or too irregular in form, are essentially similar to those of the entire animal. The pieces swim in a spiral, swerving continually toward a certain side, just as do the entire organisms. They react to chemical and mechanical stimuli by backing and turning toward the same structurally defined side, as do the entire animals. This is true of pieces forming one fourth to

one half the entire animal, whether taken from the anterior end, the posterior end, the right or left side, or the middle of the body. It is true, whether the piece does or does not bear any of the oral or other differentiated cilia.

These results establish the following facts:

1. The turning toward a structurally defined side after stimulation is not produced alone by the oral or peristomal or anterior cilia, or by any particular set of cilia, since it takes place in pieces of the body containing no specially differentiated cilia.

2. The swerving toward a certain side in the spiral swimming is not due alone to any special set of cilia, since it occurs in pieces from any part of the body.

3. The swerving toward a certain side is likewise not due to the geometrical form of the body (as to a groove on one side, such as we find in *Paramecium*), for it continues when the form of the piece is essentially altered (as by the removal of the oral groove in *Paramecium*).

4. The revolution on the long axis in a certain direction, while swimming, is not due to any special set of cilia, nor to any peculiarities of form of the body, since it occurs in pieces from all parts of the body.

5. From the foregoing paragraphs, 1–4, it must be concluded that the revolution on the long axis, the swerving toward a defined side in the spiral course, and the turning toward a structurally defined side when stimulated, are due to the method in which the cilia strike. This peculiar (one-sided) stroke is not limited to any particular set of cilia, but is shared by all the body cilia.

Division of labor is thus not marked, so far as these points are concerned, among the locomotor organs of these animals. As any portion of a crystal is organized like the entire crystal, so in the Ciliata any piece of the body, apparently, is organized so as to move and to react to stimuli in the same manner as does the entire animal.

LITERATURE CITED.

Balbiani, E. G.

'89 Recherches expérimentales sur la mérotomie des infusoires ciliés. Contribution a l'étude du role physiologique du noyau cellulaire. Recueil Zool. Suisse, T. 5.

Jennings, H. S.

'99 Studies on Reactions to Stimuli in Unicellular Organisms. III. Reactions to Localized Stimuli in Spirostomum and Stentor. Amer. Naturalist, vol. 33, pp. 373–389.

Jennings, H. S.

:00 Studies, etc. V. On the Movements and Motor Reflexes of the Flagellata and Ciliata. Amer. Journ. Physiol., vol. 3, pp. 229–260.

Jennings, H. S.

:02 Studies, etc. IX. On the Behavior of Fixed Infusoria (Stentor and Vorticella), with Special Reference to the Modifiability of Protozoan Reactions. To appear in the Amer. Journ. of Physiology, 1902.

Massart, J.

:01 Essai de classification des réflexes non nerveux. Extrait des Annales de l'Institut Pasteur. Aug. 25, 1901, 39 pp.

Pearl, R.

:00 Studies on Electrotaxis. I. On the Reactions of Certain Infusoria to the Electric Current. Amer. Jour. Physiol., vol. 4, pp. 96–123.

Pütter, Aug.

:00 Studien über Thigmotaxis bei Protisten. Arch. f. Anat. u. Physiol., Physiol. Abth , Supplementband, pp. 243–302.

Verworn, M.

'89 Psycho-physiologische Protisten-studien. Jena, 218 pp.

NOTES ON THE NATURE OF "ARTIFICIAL PARTHENOGENESIS" IN THE EGG OF PODARKE OBSCURA.

AARON L. TREADWELL.

The results obtained by Lillie[1] on the phenomena accompanying artificial parthenogenesis in *Chætopterus* led me to repeat the experiments, using *Podarke obscura*, with the idea of determining if similar results appear in this genus. I was much interested, also, in the question whether cleavage produced by chemical stimulation bears any resemblance to the normal cleavage; the latter, in this form, having been carefully followed.

That *Podarke* eggs would develop into ciliated embryos in this way was first determined by Dr. A. W. Greeley, to whose courtesy I am indebted for suggestions in the use of his method. This was treatment for one hour with a mixture of 20 c.c. of a 2½ nKCl solution + 80 c.c. of sea water. They were then transferred to sea water, and, after a variable length of time, began to divide. A solution of 22½ c.c. of 2½ nKCl solution + 77½ c.c. of sea water was also used, but gave less satisfactory results than the other. Through lack of material I did not use any other solutions, and experimented only with unfertilized eggs. This leaves my observations very incomplete, but the results thus far obtained seem worth recording.

Controls were kept in each experiment. A few of the control eggs after twelve hours or more in sea water passed into what might be termed, by a superficial observer, a "morula" stage. The cytoplasm breaks up into a great many small globules with no trace of nuclear division, the chromatin lying generally in one globule, which is usually larger than the others. These never develop further, and there was never any trace of nuclear cleavage or ciliated embryos in the control eggs. A similar breaking down into a "morula" occurs in unsuccessful experiments among

[1] F. R. Lillie, Differentiation without Cleavage in the Egg of the Annelid *Chætopterus pergamentaceus*. Archiv f. Entwick. d. Organismen, 14 Bd., 3 and 4 Hft.

the eggs treated with the KCl solution and a few usually appear among the ciliated embryos of a successful experiment.

When laid, the egg of *Podarke* passes into the stage of the first polar spindle, and remains in this condition until fertilized. It is not at all uncommon to find among late cleavage stages eggs which have escaped fertilization, and invariably they show the first polar spindle. Treatment with the KCl solution apparently does not stimulate the egg to form the polar globules, for I have not been able to find any of the latter, and in the one-cell stages found in my preparations the chromatin is either packed into a small, deeply staining mass, or scattered through the cytoplasm. Apparently, though I have not as yet sufficient evidence to demonstrate the accuracy of this conclusion, if the causes operating on the egg are such as to produce the former condition, the further change, if any occurs, is purely cytoplasmic in its character, while if the second condition appears, the egg may

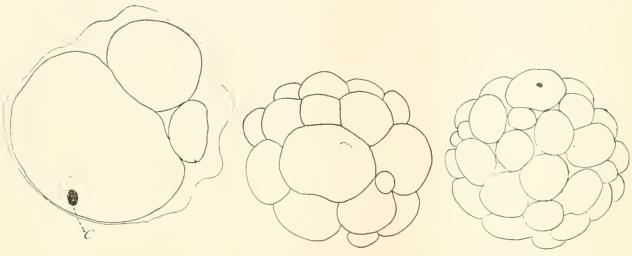


FIG. 1. Division of cytoplasm without nuclear division. *C*. Chromatin. Preserved material.

FIGS. 2 and 3. Like Fig. 1 but showing a more complete division of the cytoplasm. Preserved material.

subsequently divide by a nuclear as well as a cytoplasmic division.

As illustrations of the former case, see Figs. 1 to 3.[1] These were drawn from material preserved two hours and twenty minutes after the transfer to sea water. At this time the eggs were in active

[1] A membrane is present in all of these eggs, but is represented only in Fig. 1. The drawing from preserved material shows a wider space between the egg and the membrane than exists during life.

amœboid movement, though, as the egg membrane is closely applied to the surface of the egg, these movements are not as noticeable as in other animals. They were, however, sufficient in amount to make camera drawings impracticable. The preserved material shows that the cytoplasm is apparently broken up into distinct globules, though from the appearance presented by the living egg I think it altogether probable that they are really in communication with one another, these "globules" being really amœboid processes. As seen in the drawings, these preparations bear a superficial resemblance to a cleaving egg. There is, however, absolutely no resemblance to the normal cleavage, and a study of the stained material shows that the division is one involving only the cytoplasm, the chromatin being massed together in one of the spheres. This sphere is usually, though not always, larger than the others. (In the egg from which Fig. 3 was drawn, the chromatin, except the small bit seen in one of the upper cells, was in a large cell on the side of the egg opposite that from which the drawing was made.)

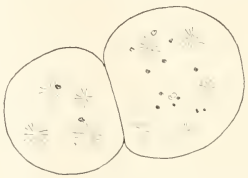


FIG. 4. "Two-cell" stage. The nuclear matter is scattered through the cytoplasm. Preserved material.

In the second of the above-described cases, cleavage involves both nucleus and cytoplasm. In Fig. 4 several asters are plainly to be seen in each cell, which leads to the suggestion that possibly it is upon the presence or absence of these that the differences in nuclear activity are dependent. Division of these eggs is apparently complete. In the two-cell stage the greatest possible variations in size between the two cells appear, the division being sometimes nearly equal, as in Fig. 4 and again very unequal. I have no reason to believe that this division is karyokinetic. It seems rather as if one of the cells is to be regarded as a lobe of protoplasm which contains some of the nuclear material, and may later become completely divided by a membrane from the other cell. Instead of two cells, a trefoil stage may appear.

What I interpret as later developments of the stage just described are shown in Figs. 6 and 7. These divisions are appar-

ently karyokinetic, but in none of them is there the slightest resemblance to the normal cleavage pattern.

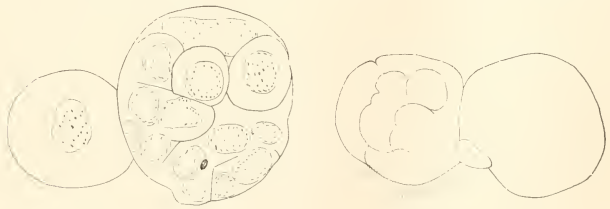
Fig. 8 is of especial interest. In about twelve hours after transfer from the salt solution to sea water, there will be found

5 6 7

FIG. 5. Trefoil stage. Preserved material.

FIGS. 6 and 7. Later development of stage shown in Fig. 4. Preserved material.

numbers of swimming embryos, looking much like the half embryo formed by destroying one blastomere of the two-cell stage. It is apparently formed from an embryo like that of Fig. 4 by a development of one cell, while the other remains undivided. Since it seems probable that the latter is to be regarded as a cell with an "extra ovate" attached, rather than a true two-celled



8 9

FIG. 8. Embryo produced by development of half of embryo shown in Fig. 4. Preserved material.

FIG. 9. Embryo like that of Fig. 8. Living material.

stage, we might say that here either the extra ovate or its fellow developed, while the other part remained undivided. These divided portions put out cilia, and swim about in the water, carrying the undivided portion with them. Later, they break away

from the undivided portion and swim independently, forming "dwarf embryos." So far as I can discover, their cleavage has no resemblance to the normal. Their abnormal character is also indicated by their nuclei, which have a very characteristic appearance, resembling closely those of abnormal eggs which occasionally appear among a normally fertilized lot. The nuclei are much larger and stain much more intensely than the normal ones. Fig. 9 is from a living egg of this sort, and shows the arrangement of the cilia.

Besides these ciliated embryos are others, showing no indication of cleavage, but with well-developed cilia. Fig. 10 shows

10 11

FIG. 10. Ciliated, unsegmented embryo. Living material.
FIG. 11. Unsegmented embryo formed by fusion. Living material.

one of these where the whole embryo is very much distorted, but with a well-marked tuft of cilia. Staining with aceto-carmine after the above drawing was made, gave no indication of cleavage in this embryo.

Fig. 11 shows a case of fusion of at least two eggs. This was from an embryo slowly rotating around the end *A* as a pivot. I was unable, however, to discover the distribution of the cilia causing this movement.

Fig. 12 shows a still more interesting condition. Here, also, there is apparently a fusion of at least two cells, but there is no trace of cleavage. At *n* is possibly a nucleus. Around one portion of the fused mass is a ring of cilia, occupying very much the position, with respect to the cell, of the prototroch in its relation to the trochophore. Not only are the cilia present, but around the embryo, underneath the ciliated band, is an area free

from the ordinary pigment of the rest of the cell, but containing granules of a faint yellow color, agreeing in this respect exactly with the prototroch band of the normal trochopore. In this embryo there is, then, not merely a differentiation—without cleavage—of cilia, but of the characteristic protoplasm accompanying these cilia—or, in other words, we have here, in the unsegmented embryo, not merely a differentiation of cilia, but a differentiation of *prototroch cilia*. Later, fused masses like those just described are apt to break down, setting free the ciliated fragments, and by the twentieth hour the culture will be swarming with ciliated fragments derived from this source and from the breaking away of the "dwarf" described above. These, of course, have not even a superficial resemblance to normal embryos.

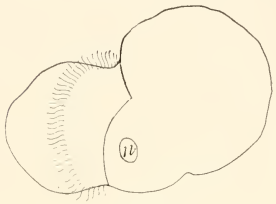


FIG. 12. Unsegmented embryo formed by fusion with well-differentiated band of cilia. Living material.

Fusion phenomena are rare in *Podarke* as compared with *Chætopterus*, the largest fused masses I have found containing not more than six eggs.

SUMMARY.

1. After treatment with 2½ nKCl solution, eggs of *Podarke obscura* exhibit various cleavages and pseudo-cleavages.

2. The pseudo-cleavages involve only the cytoplasm and not the nucleus, and have no resemblance to the normal cleavages.

3. The cleavage involves both nucleus and cytoplasm, and may give rise to a ciliated embryo. The cleavage in this case, however, does not resemble the normal.

4. Ciliated embryos may arise without cleavage. The differentiation may be carried very far in such cases. This is especially noticeable in the character of arrangement of cilia.

5. Fusion of embryos may occur, but in the solution mentioned, much fewer cells unite than in, *c. g.*, *Chætopterus*.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASS., Sept. 5, 1902.

BIO BULL V. III 1902 : 241-244

HABIT-FORMATION IN THE GREEN CRAB, CARCINUS GRANULATUS.

ROBERT YERKES,

MARINE BIOLOGICAL LABORATORY, WOODS HOLL, MASS.

In his excellent paper[1] on the structure of the nervous system of *Carcinus mœnas* Albrecht Bethe describes certain experiments the results of which he believes to justify the conclusion that the green crab does not profit by experience, and therefore does not possess "psychischen Qualitäten."

The following are two of the experiments by which Bethe tested the ability of *Carcinus* to learn. A crab was placed in an aquarium which contained an *Eledone* in the darkest corner. Immediately the crab scurried off into the dark region and was seized by the *Eledone* and drawn under its mantle. The experimenter then interfered by quickly freeing the crab from its enemy and returning it to the opposite end of the basin. Again the crab hurried into the shaded region, was seized, freed and returned to the light. This experiment Bethe says he repeated with one individual five times and with another six times without obtaining any evidence that the crabs were learning to avoid the danger. In another test crabs in a basin were baited with meat, and each time they tried to get the food they were seized and maltreated by the experimenter. Thus an attempt was made to teach them that meat, under certain conditions, meant danger. But in this test also several repetitions of the experiment failed to teach the crabs to avoid the danger.

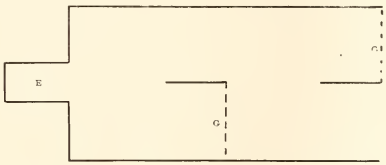
Bethe's tests are unsatisfactory because in each he attempts to inhibit an instinctive action. In the first test the animal naturally sought to hide in the dark; and it would be absurd to expect to modify this instinct by giving the animal five, six or even twenty-five experiences of the disagreeable consequences of going into the neighborhood of the *Eledone*. And in the second test the reflex food-seeking reaction to a particular kind of chemical

[1] Bethe, Albrecht: Das Centralnerven-system von *Carcinus mœnas*. II. Theil., Arch. f. Microscop. Anat., Bd. 51, 1898, S. 447.

stimulation stood in the way of the establishment of new modes of response.

Bethe should not have expected a few experiences to modify or inhibit such fundamentally important reactions as those to light and to food. Almost any reaction can be modified if time enough be devoted to that end, but it is as unlikely that a crab would learn to avoid the dark or to inhibit its food-taking reaction after a half dozen harmful experiences in a certain situation as it is that the grasshopper would learn to inhibit its jumping by a few experiences in a glass box. For the modification of such reactions hundreds, not tens, of experiences are necessary.

In view of the obvious unfairness of Bethe's tests of *Carcinus* I have made a further study of the ability of the American species of *Carcinus*, *C. granulatus*, which is similar to the European form, *C. mœnas*. My effort has been to determine whether



Floor Plan of Experiment Box. Scale one eighth.

the animals can learn (1) a simple labyrinth path to their food, and (2) to avoid the unpleasant experience of being captured in a hand-net and taken from the aquarium.

The crabs were kept in an aquarium about 60 cm. long, 38 cm. wide and 35 cm. deep, over which an experimenting box 40 cm. × 20 × 12 was placed so that its exit passage just touched the surface of the water. The accompanying cut which is the floor plan of the box, shows, in addition to the entrance passage *E*, in which the crab was placed by the operator at the beginning of each experiment, two alleys which were closed by glass plates *G*, *G*. Experiments were made to discover whether the green crab would learn to avoid these blind alleys in its efforts to get back to the water of the aquarium, and if so how quickly it would learn.

The only motive relied upon for the return of the crab to the aquarium from the experimenting box was its desire to get into the water.

For two weeks several crabs were given on an average four trials per day in the labryrinth. At the beginning of the work they frequently wandered in the entrance passage and blind alleys several minutes before they succeeded in escaping, but after twenty or thirty trials most of them were able to escape directly. I was unable to get any satisfactory quantitative expression of the rapidity of learning because of the great variation in strength of the animal's desire to get back to the water; at one time it would move about constantly until it found the exit, at another it would rest quietly in the passages for long periods. In the case of an animal which, for a month, was given two trials per day in the labyrinth, the escape at first took about five minutes, and at the end of the period about ten seconds.

At first the crabs when placed in the box and poked with a stick scurried off into a corner and took a defensive position; whereas, after a few escapes from the box, they made directly for the exit as soon as they were threatened with a stick.

Even fifty experiences, in case of most of the individuals studied, did not result in the formation of a perfect habit.

Further tests of the ability of the green crab to learn were made by placing a wire-screen partition in the middle of the aquarium and allowing the animals to find their way through an opening in the screen to food on the opposite side. Since the crabs tend to follow the edge of such a screen, the opening, 10 cm. x 10, was made in the middle of the screen. It was therefore about 12 cm. distant from the sides, top and bottom of the aquarium.

In this test each crab was given a single experience per day. To provide the animals with a motive for seeking to pass beyond the screen, at the beginning of each trial a piece of fish meat was placed in the corner of the aquarium diagonally opposite to that from which the crab started. The meat having been placed, a crab was put into the aquarium, and a record was kept of its wanderings in search of the food, and of the time occupied in finding it.

The following are the results of ten trials with two animals.

Number 1.		Number 2.	
Trial.	Time in Minutes.	Trial.	Time in Minutes.
1	7	1	23
2	4	2	10
3	$3\frac{1}{2}$	3	$4\frac{1}{2}$
4	4	4	3
5	2	5	$2\frac{1}{4}$
6	1	6	$6\frac{1}{2}$
7	1	7	7
8	$1\frac{1}{2}$	8	$1\frac{1}{4}$
9	1	9	$1\frac{1}{5}$
10	$1\frac{1}{4}$	10	$\frac{1}{2}$

Crab number 1 almost invariably moved directly toward the meat and searched for an opening in the screen along the side of the aquarium nearest the meat, thence it would move to the middle of the screen and find the opening. Number 2 just as uniformly followed the edge of the screen, climbing up one side, along the top, down the other side, and across the bottom ceaselessly until it chanced to get near the opening.

The paths for the first trials were complex and devious, but in the later trials they were almost direct lines to the meat.

There was little evidence of the use of vision in the finding of the opening in the screen. The animals seemed to be directed chiefly by the stimulus from the food.

Additional evidence of the ability of *Carcinus* to profit by experience is furnished by the following observation. It was the habit of the experimenter to catch the animals in a hand-net, take them from the water and use them for various experimental purposes. At first the crabs did not react otherwise to the approach of the experimenter with the net than to other large objects, but after a few days it became much more difficult to capture them for they had learned to avoid the net.

Vol. III. *November, 1902.* *No. 6.*

BIOLOGICAL BULLETIN.

THE FOLLICLE SACS OF THE AMPHIBIAN OVARY.

HELEN DEAN KING.

In 1738, Swammerdam (6) stated that just after the eggs of the frog have been laid, one can find on the inner wall of the ovary, "some empty and very delicate membranes which had served to invest the eggs that had been already discharged from the ovary. . . . These particles were most beautifully interwoven with blood vessels to which they were fixed as to so many stalks." Swammerdam does not give the origin of these membranes nor does he state how the eggs pass from the ovary into the body cavity.

Over one hundred years later, Thompson (7) wrote that the ovarian egg of the frog and toad "is surrounded by a thin vascular sac formed by the dilation of the ovisacs which hang into the general ovarian cavity. This capsule or ovisac is attached to the rest of the ovarian substance by a broad band rather than by a narrow pedicle; and when the yolk or ovarian ovum is mature, it escapes from the ovisac by the formation of an aperture in the remote or free side of this capsule, somewhat in the same manner as occurs in the calyces of the bird, but with a wider aperture. Through the apertures of the general ovarian capsule the numerous ova pass into the abdominal cavity." In thus stating that the ovarian eggs first fall into the general cavity of the ovary and later pass into the body cavity through openings in the ovarian wall, Thompson is in agreement with earlier writers, Rathke (4) and Lereboullet (3). More recently this view has been opposed by Brandt (1) who examined the outer surface of the ovary of *Rana temporaria* as the eggs were about to pass into the body cavity and found a round hole above each egg through which a larger or smaller part of the egg protruded. This discovery led him to state: "Es ist mithin klar, dass für jedes sich lösende Ei

eine besondere Austrittsöffnung in die Peritonealhöhle hergestellt wird."

According to Brandt, the ovarian egg of *Rana* is surrounded by membranes whose origin he describes as follows: "Die Follikel des Ovariums dürften dadurch entstehen, dass die einzelnen sich vergrössernden Eianlagen von den benachbarten, sich proliferirenden und abplattenden Elementen, sowie von den Bindegewebselementen der Ovarialwandung umwachsen werden." These membranes are said to form the follicle sacs that are found on the inner wall of the ovary after the eggs have passed into the body cavity, but no mention is made of their later history. During his investigations, Brandt carefully injected colored fluids into the ovary just after the eggs had been laid and found that none of the fluid ever came through the walls of the follicle sacs. He was thus able to show conclusively that there is no communication between the cavity of the ovary and the body cavity through the walls of the follicle sacs. The latter have but the one opening which leads outward into the body cavity.

The investigations recorded in the present paper were made to supplement the work of Brandt and others with a more detailed description of the origin and fate of the follicle sacs of the amphibian ovary. Observations were made on three different amphibians, *Bufo lentiginosus*, *Rana palustris* and *Hyla*. The general description and the drawings (made with a camera lucida) are taken entirely from *Bufo lentiginosus* as I had a much more complete series of this species. As the relatively few stages of *Rana* and *Hyla* at my command confirmed the results obtained on *Bufo* in every respect, a separate description of the follicle sacs in these two amphibians seemed unnecessary and is, therefore, omitted. In preparing the material for study, small portions of the ovary were fixed in corrosive-acetic, and then stained on the slide either with iron-hæmatoxylin, or with a mixture of Lyon's blue and borax carmine, as described in a previous paper (King, 2).

The ovary of *Bufo lentiginosus* consists of several distinct compartments, agreeing in this respect with the ovary of various species of frogs, according to the observations of Rathke, Lereboullet, Brandt and Schultze (5). The walls of these compart-

ments are made up of two membranes which, as a rule, are separated some distance, although occasionally they lie so close together that they appear to be completely united. The outer membrane is single, compact, and of nearly uniform thickness throughout the entire ovary. The inner membrane, on the contrary, appears in some places to be very similar in structure to the outer one; in other parts of the ovary it is found to be composed of two thin layers which always lie close together and are connected at frequent intervals. Cells with very large nuclei are closely applied to the outer and inner surfaces of these membranes and are also present in the spaces between them. (Figs. 3–6, *O. W.*)

The eggs develop between the two membranes composing the wall of the ovary. As they increase in size, they press against the inner membrane of the ovarian wall and cause it to project more and more into the cavity of the ovary. Part of the ovarian

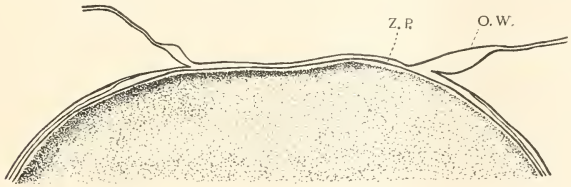


FIG. 1. A portion of an ovarian egg showing the manner of its attachment to the wall of the ovary. Z. P. Zona pellucida; O. W., outer wall of the ovary. Zeiss. obj. A. Oc. 4.

wall comes, therefore, to form a sac that incloses the egg and supplies it with nourishment as long as it remains in the ovary. When the egg is still quite small, there is formed, close to its outer surface, a true egg-membrane, the "zona pellucida" or "chorion," which is thick and seemingly homogeneous in structure (Fig. 2, *Z. P.*). This membrane which is formed, probably, by the numerous follicle cells that are scattered all over the surface of the egg, surrounds the egg during its growth and maturation periods and is invariably found around all eggs taken from the body cavity. Numerous cells and occasionally blood corpuscles (Fig. 2, *C.*) are found between and under the two layers

of membrane which form the outer covering of the egg. These cells, which are usually oblong in shape, contain a small amount of faintly staining, granular protoplasm, and a very large, rounded nucleus with one nucleolus. The chromatin is either collected in masses along the nuclear wall, or it is in the form of a more or less regular network scattered throughout the nucleus.

Ordinarily, when the egg of *Bufo lentiginosus* is ready to leave the ovary, it has a diameter of about 1.5 mm. and from 0.60–0.70 mm. of its surface is in contact with the outer membrane of the ovarian wall (Fig. 1). In the great majority of cases, the egg is attached at the equatorial region as in Fig. 1 ; but it is not uncommon to find among the eggs thus attached, one with its black or its white pole against the outer membrane.

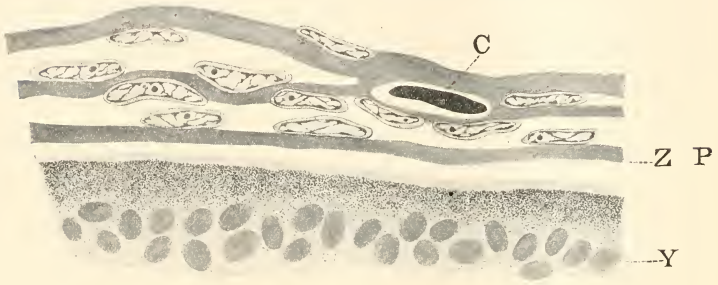


FIG. 2. Part of an ovarian egg highly magnified, showing the structure of the membranes surrounding the egg. C, blood corpuscle ; Y, yolk ; Z.P., Zona pellucida. Zeiss apoc. 2 mm. Oc. 8.

By what means the ovarian wall is ruptured and the eggs set free into the body cavity is not known. This process cannot be connected with the breaking down of the germinal vesicle, because the nucleus degenerates and the first polar spindle is formed some time before the egg leaves the ovary, as I have shown in a previous paper (2); neither can it be due to the way in which the eggs are attached to the ovarian wall, for, as I have stated above, the mode of attachment varies with individual eggs. Apparently the eggs must reach a definite stage of development before they break away from the wall of the ovary, because, although I have

sectioned a large number of eggs taken from the body cavity, I have never found a single one in which the polar spindle was not fully formed with the chromosomes arranged along the spindle fibers. Brandt suggests that there may be an active con-

FIG. 3. Longitudinal section of a follicle sac just after the egg has passed into the body cavity. C, blood corpuscles; O. W., ovary wall. Zeiss obj., D. Oc. 4.

traction of the follicle sac when the egg is ready to leave the ovary, and that this contraction stretches the base of the follicle and causes a rupture. How such a contraction takes place without the presence of muscles in the walls of the follicle sac is not at all clear. It seems to me that the rupture of the ovarian wall is more likely to be due to some activity on the part of the egg

itself. Possibly there is a slight increase in the size of the egg at this time which produces sufficient pressure on the surrounding membranes to cause a break at the weakest point. This would seem to be where the egg is attached, because here there are only two membranes over the egg instead of three (Fig. 1). This suggestion, however, fails to account for the rare cases where the egg breaks through its membranes and falls into the cavity of the ovary. Such eggs never leave the ovary; sooner or later they undergo degenerative processes and are gradually absorbed, as are also the few eggs that do not leave the mem-

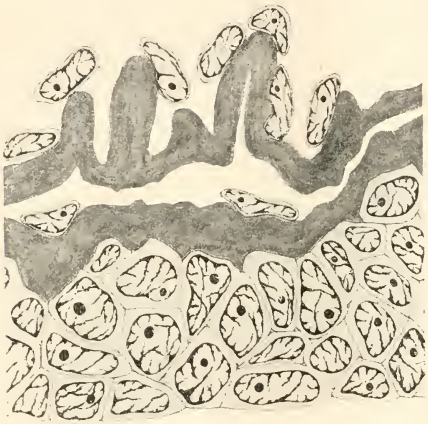


FIG. 4. Part of the follicle sac shown in Fig. 3 more highly magnified. Zeiss apoc. 2 mm. Oc. 8.

branes at all but remain attached to the ovarian wall. In the latter case the eggs are almost invariably smaller than those taken from the body cavity, and, therefore, retarded development may explain their failure to undergo normal processes.

On spreading out a piece of the collapsed ovary after the eggs have passed into the body cavity, one can plainly see the numerous follicle sacs without the aid of a microscope. These sacs vary in length from 0.36 mm. to 0.54 mm., and their walls are very much wrinkled and folded. Although from 0.60 to 0.70 of

the mature ovarian egg is in contact with the outer membrane of the wall of the ovary, I have never found the opening of an empty follicle sac to be more than 0.38 mm., the average being 0.20 mm. This shows that the edges of the opening tend to close in as soon as the egg has passed out of the ovary.

On examining a longitudinal section of an empty follicle sac (Fig. 3), one finds that the two layers of which its walls are composed are united at frequent intervals, and that, on the wall and in the spaces between the layers, there are numerous cells.

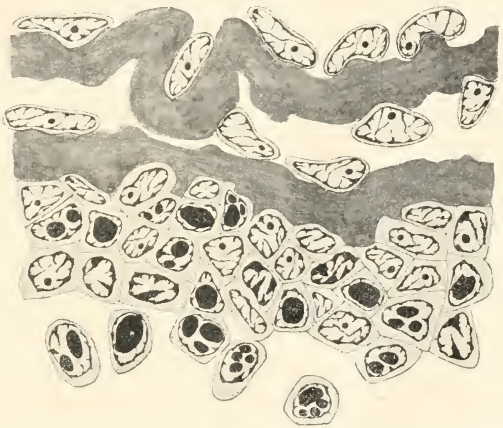


FIG. 5. A portion of the wall of a follicle sac after degenerative changes have begun. Zeiss. apoc. 2 mm. Oc. 8.

Sections of the small blood vessels which supplied the eggs with nourishment are also found occasionally (Fig. 3, *C*). The cavity of the follicle sac is lined with cells, several layers deep in places, particularly at the closed end of the sac. These cells are doubtless the ones that lay between the zona pellucida and the outer covering of the egg at an earlier period, and their confinement in a much smaller space must explain why they appear so numerous, as I have never found any evidence of cell division at this time.

A part of the wall of a follicle sac more highly magnified is shown in Fig. 4. The cells lining the cavity of the sac are seen

to be very closely packed together, and there is rarely an intercellular space to be found. These cells have the same characteristics as those shown in Fig. 2; namely, a very large nucleus containing one nucleolus, a small amount of granular protoplasm, and chromatin partly in the form of a network, and partly in masses scattered along the wall of the nucleus.

On examining the ovary of a toad killed about three weeks after the eggs had been laid, one finds that the openings of the follicle sacs into the body cavity have closed, and that the outer membrane of the ovarian wall is again continuous. Each follicle sac appears at this time as a blind pouch projecting from the inner side of the ovary. The cells lining the cavity of the sac are beginning to show the degenerative changes that are very marked in the ovaries of toads killed the latter part of May. The most noticeable changes are those which are taking place in the nucleus (Fig. 5). Although at this time the nucleus is still round or oval, its outline is somewhat more irregular than it was at an earlier period, and most of its chromatin is collected in from one to four rounded masses that stain black with iron-hæmatoxylin and a deep red with the borax carmine and Lyon's blue mixture used. The protoplasm of the cells appears very granular, and it stains so feebly that it is often very difficult to determine the cell outlines. Such changes, as a rule, are first apparent in the innermost cells that soon break away from the rest and become scattered throughout the cavity of the follicle sac. These free cells never show any pseudopodia; on the contrary, they are more rounded than are the cells still attached to the walls of the follicle sac, and it is therefore impossible to determine whether they have the power of independent movement or not.

Although I have examined the ovaries of many toads killed during the month of June, I have not been able to determine whether the cells lining the follicle sacs are destroyed by the leucocytes, as are the eggs that fail to leave the ovary, or whether they disintegrate and are absorbed by the cells of the ovarian wall. In all the toads killed at this time, either the cells within the follicle sacs were undergoing degenerative changes similar to those shown in Fig. 5, or else the cavity of the follicle sac was nearly obliterated and no traces of the cells could be found. It

is evident that the final stages in the disappearance of these cells take place very quickly and that a large amount of material would be necessary in order to trace their entire history. A longitudinal section of one of the follicle sacs found in the ovary of a toad killed on the fifth of June is represented in Fig. 6. The sac measures 0.13 mm. in length and is, therefore, about one third its original length. The walls of the sac are thinner and

FIG. 6. A longitudinal section of a degenerating follicle sac taken from the ovary of a toad killed on the fifth of June. Zeiss apoc. 2 mm. Oc. 4.

much less folded than they were at an earlier period, and its cavity has nearly disappeared. Numerous cells are found closely applied to the walls of the sac at this time, but they show no degenerative changes, and are undoubtedly concerned in the absorption of the follicle sac itself. Much smaller structures of the same general character as that shown in Fig. 6 can be seen in the ovaries of toads killed the latter part of June; but after this time no traces of them can be found.

The follicle sacs, having served the important function of attaching the eggs to the walls of the ovary and of supplying them with nourishment during their growth period, are of

no further use after the eggs pass into the body cavity. They therefore undergo rapid degeneration and absorption to make room for the young eggs that are in the process of development.

LITERATURE.

Brandt, Alexander

1 Fragmentarische Bemerkungen ueber das Ovarium des Frosches. Zeitschr. f. wiss. Zoöl., Bd XXVIII., 1876.

King, Helen Dean

2 The Maturation and Fertilization of the Egg of Bufo lentiginosus. Journ. Morph., Vol. XVII., 1901.

Lereboullet, A.

3 Recherches s. l'Anatomie des Organes genitaux d. animaux vertébrés. Nova Acta Acad. Leop. Car., t. XXIII., 1851.

Rathke, H.

4 Beiträge zur Geschichte der Thierwelt III. Neueste Schr. d. Naturf. Ges. in Danzig, Bd. I., 1825.

Schultze, O.

5 Untersuchungen über die Reifung und Befruchtung des Amphibieneies. Zeitschr. f. wiss. Zoöl., Bd. XLV., 1887.

Swammerdam, J.

6 Biblia Naturæ. Leydæ, 1738. Trans. by Thos. Flloyd, London, 1758.

Thompson, Allen

7 Ovum. Todd's Cyclopædia of Anatomy and Physiology, Vol. V., 1859.

THE MOVEMENTS OF THE ENTEROPNEUSTA AND THE MECHANISM BY WHICH THEY ARE ACCOMPLISHED.

WM. E. RITTER.

The observations upon which the present paper is based were made, so far as they pertain to the living animal, upon two species of the group found on the Coast of California and Washington. These are *Balanoglossus occidentalis* Ritter (MS.) and *Dolichoglossus pusillus* Ritter (MS.). The first mentioned is abundant at Puget Sound, and is found, though in small numbers, at San Pedro, California. The second is widely distributed along the Coast of California and is exceedingly abundant at San Pedro, where it lives side by side with the first.

My study of the movements have been made mostly on *D. pusillus*. This small species is so abundant in limited areas at San Pedro that a single shovelful of the sand in which it lives may contain several dozen. Its place of greatest abundance is a large sand flat, exposed at low tide, just inside the mouth of the inner harbor at Timm's Point. The sand in which the animals live is compact and homogeneous and contains much fine argillaceous material, the whole being blackened by decaying organic matter. In some places the creature is found among the roots of scattered patches of eel grass. Although the species is closely related to *D. Kowalevskii* of the Atlantic Coast of the United States, it is considerably smaller. It never produces the coiled castings so characteristic of its eastern relative. In fact, I have failed to discover any marking on the surface of the sand that can be relied upon to reveal the presence of the creature. The absence of the casts which are such a convenience to the collector is, however, compensated by the animal's habit of the protrusion of the proboscis from the ground. At low tide large numbers of the orange-colored proboscises may be seen with the tips only, or sometimes with nearly the entire organ, lying flaccid on the sand. A slight jarring of the ground causes a prompt withdrawal of the exposed part. Professor B. M. Davis, of the Los Angeles State

Normal School, first called my attention to the fact that the protrusion of the proboscis occurs only while the sand is uncovered during low tide ; that withdrawal takes place almost immediately upon the incoming of the tide. My own observations confirm this. I have several times searched the ground in which the animals are abundant, when it was covered by a few inches only of still, clear water, and have never under such conditions seen a single extruded proboscis. The meaning of this habit is not clear.

Movements of both boring and locomotion are effected in the enteropneust through a combination of ciliary and muscular action. When crawling about on the surface of objects, which it does with considerable facility, the ciliary method is chiefly, though by no means exclusively, depended upon. When boring, or moving up and down its canal, muscular action is on the other hand most used.

The best way to observe the movements is to allow the animals to bury themselves in sand contained in a glass vessel. Under such circumstances they are certain to move along the inner surface of the glass if the vessel be small (a test-tube or homeopathic vial, for example, I have found favorable), for a portion of the time, and the whole operation may then be easily watched.

By far the most vigorous activity in any portion of the animal, both ciliary and muscular, goes on in the proboscis. The ciliary action is seen to best advantage when the animal is moving about on a sandy surface on which there are small elevations with which the tip of the proboscis comes in contact now and then ; for the creature does not readily bring its proboscis tip into contact with a perfectly even surface on which it may rest. As the end of the proboscis touches the slope of a sand hillock, a perfect flood of sand grains is at once started which soon wholly encircles the organ and passes rapidly back to the collar. Arrived here the rapidity of the flow is greatly lessened so that if the picking up of grains at the tip be prevented a girdle of sand is soon formed around the collar.

If, on the other hand, the acquisition of grains be not interrupted the whole proboscis and collar become coated by a continuous sheath of sand which gradually extends backward until

the whole animal is covered, the sheath being scarcely more than one or two grains in thickness. The backward movement of the sand on the collar and body is effected chiefly by the pushing force of the ciliary action of the proboscis, though a weak ciliary action on the collar and thorax also contributes to the result, as does likewise muscular contractions of the proboscis. The prolific secretion of mucus particularly by the proboscis and collar serves to agglutinate the sand grains, to produce the sheath above mentioned. This sheath forms a well-defined tube when produced under the conditions here described so firm as to support itself in pieces of considerable size when pulled off and held up in the water.

Simultaneously with the ciliary action the animal is undergoing almost constant contortions through muscular action; but the true nature and significance of the motions of this kind are not seen until the creature is observed in the processes of burrowing. Here the meaning of a characteristic movement of the proboscis which is constantly noticed when the animal is not buried is readily seen. I refer to the contraction waves that move along the organ from tip to base, but which often remain stationary in the form of great blebs.

When in its burrow these blebs act chiefly as hold-fasts by which, through the contraction of the longitudinal muscles of the proboscis and collar, the whole body is drawn forward. The muscles most concerned in this are first those of the proboscis; second, the radio-longitudinal muscles of the collar; and third, the longitudinal muscles of the thorax-abdomen.

The proboscis tip is driven forward in making a new burrow partly by the action of the cilia, and partly by the contraction of the layer of circular muscle fibers, the former apparently playing the more important part.

The wave motion so characteristic of the proboscis does not appear on the collar, thorax, or abdomen, so that it plays no part in the advancement of these portions of the body. The collar shortens and elongates without great change in diameter, though the anterior rim is sometimes narrowed down considerably, the possible change in length of the part being nearly or quite one half its entire length in the fully extended state. The shortening is un-

doubtedly effected almost wholly by the contraction of the radio-longitudinal muscles. The extension is probably accomplished, as Spengel believes, by the inflation of the collar cœlom with water through the collar funnels.

Observation on the movements of the animal furnishes little satisfaction to one's quest after a reason for the existence of the peduncle that joins the proboscis and collar. At the same time, however, such observation does emphasize the importance of the slender isthmus's being both strongly constructed in itself and also firmly anchored at the ends to the larger parts of the body into which they are inserted. As the animal draws its long body about by its proboscis, it frequently looks as though the slender peduncle would be pulled out by the roots, particularly at its collar end. One naturally conjectures that the existence of the peduncle is to increase mobility at this particular place, and this perhaps is its significance, though it is not clear that the peduncle is in reality more flexible than the proboscis, especially when the latter is perfectly flaccid, for I have seen this part when the animal was in its canal double back upon itself so as to bring the two limbs into contact throughout their length, the tip of the organ being carried back to its base. The collar and thoracic portions of the body, however, are always, and the proboscis when muscular contractions are in progress, relatively rigid; and it is probably under these conditions that the mobility assured by the peduncle, finds its importance.

Having now the facts before us relative to the movements of the animal, we may consider the anatomical structures upon which these depend.

The proboscis possesses a typical dermo-muscular tube and its movements are essentially of the wave form usual to this type of musculature. The close resemblance between the movements of the enteropneust proboscis and those of the nemertean may be especially mentioned.

The thorax-abdomen, also, may be regarded as possessing the dermo-muscular tube, though here circular muscles are either altogether wanting or are very imperfectly developed, so that the wave movement does not here take place. Furthermore the longitudinal muscles are much stronger in the ventral than in the

dorsal half of the body in these regions. Ventral curvature as well as fore-and-aft shortening is consequently a general result of the contraction of these muscles.

The chief interest in the myology of the animal centers in the collar and peduncle. Here we have no dermo-muscular tube, but, on the contrary, a musculature of a fundamentally different type. The muscles, both longitudinal and circular, of the dermo-muscular tube are always, it will be recalled, strictly somatic, *i. e.*, they belong to the *body wall.* The chief mass of muscles of the collar and peduncle, viz., the radio-longitudinal muscles, while attached to the body wall at one end, at the other are on the other hand attached to the *notochord* and *nuchal skeleton* mainly, but partly also to the *wall of the esophagus.*

The full details of the origin, insertion, and relations of these large muscles are too complicated to admit of being fully set forth otherwise than by an elaborate, well-illustrated description; but an understanding of the most important facts may be given by a brief presentation, particularly if reference be made to Spengel's monograph. Figures 11, Pl. 14, and 20, 28, 32 and 34, Pl. 15, *lmi.* marking the muscles under consideration, are especially clear and instructive in this connection.

The two muscle masses are bilaterally situated and are in general coextensive in length with the collar and peduncle together. Their origins are on the sides of the combined notochord and nuchal skeleton anteriorly, *i. e.*, in the peduncle; and on the esophageal notochord and esophagus itself posteriorly, *i. e.*, in the collar (compare Figs. 4 and 7, *i. l. c. m.*, Pl. VII., of the author's paper on *Harrimania maculosa*[1]).

From these extensive origins the fibers extend backwards and outwards, some to become inserted into the outer edges of the septum separating the thoracic and collar cœloms; some, with a more oblique course, to insert into the ectoderm or the underlying connective tissue, along the postero-lateral portions of the collar; and still others at the anterior end of the collar, with a course predominantly radial, to insert into the ectoderm of the

[1] Papers from the Harriman Alaska Expedition, II. *Harrimania maculosa*, a new genus and species, etc. *Proc. Wash. Acad. of Sciences*, Vol. II., Aug. 20, 1900, p. 111.

anterior rim of the collar. By the contraction of these muscles when the proboscis acts as a fixed point through the pressure of the blebs above described against the sides of the burrow, the collar and, of course, the whole pharyngeal-abdominal portion of the body, are drawn strongly forward. Following, or coincidently with, this movement, contraction of the longitudinal muscles of the last-mentioned parts themselves takes place which contributes still further to the advancement of the creature as a whole. Circular somatic muscles are practically wholly wanting in the collar. A few fibers have been described in some species, but I am unable to recognize any excepting a few in the anterior rim, in any of the species examined by me.

From the account here given it is seen that we have in these animals *a system of locomotor muscles acting on an axial skeleton, derived in large part from the digestive tract.* That the combined notochord, consisting of the proboscis and esophageal portions, and nuchal skeleton, must be interpreted as a *unit of structure with the office of an axial skeleton*, I hope to be able to show conclusively in my forthcoming monograph of the Enteropneusta of the Pacific Coast. I imagine, however, that the proposition will be readily granted by most zoölogists, particularly if the facts here briefly set forth be considered along with others that may be gathered from previous publications on the anatomy and development of these animals.

If now the considerations relative to the skeleton and muscles be regarded from the comparative standpoint, the most striking thing that comes to view is the trenchancy with which they separate the enteropneusta from all the invertebrata. Nowhere in all the variety of organization represented by this subkingdom do we find locomotion accomplished by *muscles attached either to the intestinal tract or to derivatives of it.* But the trenchancy with which the structural relations here considered separate the enteropneusta from the invertebrata is no greater than that by which they connect them with the chordata.

An axial skeleton derived primarily from the intestinal tract and serving as the attachment of muscles of locomotion is, as is fully recognized, one of the very essences of chordate organization; and in spite of the difficulties in the way of establishing a

complete homology point by point between the axial skeleton (as I have defined it) of the enteropneust with its appended radio-longitudinal muscles of the collar, and the axial skeleton of amphioxus with its attached system of muscle plates, I can but believe that the two systems must have had, far back, a common origin.

But the problem is too large to admit of extended discussion in the present communication. My immediate purpose will have been attained if I shall have called the attention of zoölogists to the undoubted fact, as it seems to me, that the *muscular relations in the collar region of the enteropneusta must receive consideration from a point of view that has not hitherto been held, in all future attempts to elucidate the kinships of this group of creatures.*

UNIVERSITY OF CALIFORNIA,
August 28, 1902.

EMBRYONIC AND REGENERATIVE DEVELOPMENT IN PLANARIANS.

CHARLES RUSSELL BARDEEN,

MARINE BIOLOGICAL LABORATORY, WOODS HOLL, MASS.

The term "regenerative development" may be applied to the formation of a new from a part of a preëxisting individual whereby certain structures are carried over essentially unchanged from parent to daughter. In the following paper regenerative and embryonic development in *Planaria maculata* are compared.

EMBRYONIC DEVELOPMENT.

The classical account of the embryonic development of planarians is given in the excellent paper of Iijima, published nearly twenty years ago.[1] Iijima studied more especially the development of *Dendrocœlum lacteum*. The development of *Planaria maculata* seems to be essentially similar. With the early embryonic stages down to the formation of the "embryo-pharynx" we are not at present concerned. It is the transformation of the spherical embryo, filled with yolk cells, into a worm of normal structure and proportions that is of special interest from the standpoint of regenerative development.

At the period when the imbibition of yolk cells begins to be active the planarian embryo is spherical in form. It is surrounded externally by a well-marked membrane formed of greatly distended, flat epithelial cells, shown in section in Fig. 2, *A*. Between this membrane and the yolk cells which fill the central cavity is a reticular substance in which are scattered large cells with nuclei containing one or more nucleoli. These cells are represented by the larger bodies in the body-wall shown in Fig. 2, *A*. One of them is shown in Fig. 4, *a*. The nucleus is immediately surrounded by a granular mass of protoplasm. The reticular substance of the body wall probably represents the

[1] Iijima: "Unters. uber die Bau u. die Entwicklungsgesch. d. Süsswasser-Dendrocœlen (Tricladen)," *Zeitschrift f. Wissenschaftliche Zoölogie*, Vol. XL., 359, 1884.

syncytial "ectoplasm" of these cells. Cell division at this stage is mainly by mitosis. In addition to the cells just described many small nuclei, granular masses, and vacuoles are embedded in the substance of the body wall. These bodies I take to be remnants of yolk cells. Most of the small nuclei show marked evidences of disorganization. Some of the large cells of the body wall lie flattened against the yolk cavity but I can find no evidence at this stage of a differentiation between entoderm and mesoderm cells like that described by Iijima for *Dendrocœlum*. The embryo-pharynx corresponds closely in structure to that described by Iijima.

As the embryo becomes distended by taking in yolk cells the body wall becomes thinner and the character of the cells lying in the reticular substance is altered. The nucleus becomes more densely granular and division takes place mainly by amitosis (see Fig. 4, *b*). The protoplasm of these cells varies in granulation and in staining power, but I have been able to distinguish no clearly defined classes of cells. Rapid cell division gives rise to a smaller variety of cell shown in Fig. 4, *c* and *d*. These cells exhibit indications of amœboid movement, as shown in Fig. 4, *d*. In these smaller cells mitotic division prevails, though amitosis is also present.

The hitherto spherical embryo now becomes flattened dorso-ventrally and elongated in the antero-posterior axis. This change in form is accompanied by a more rapid cell-multiplication in the ventral than in the dorsal body wall. The most rapid cell-multiplication takes place on the postero-ventral wall just below the embryo-pharynx (see Fig. 2, *B*). It is from this mass of cells that the permanent pharynx is developed. Iijima says that in *D. lacteum* the permanent pharynx is developed in the region of the embryo-pharynx after the disappearance of the latter. The relations between embryonic and permanent pharynxes in *Planaria maculata* were first discovered by Winterton Curtis and are described by him in an article shortly to appear in the *Proceedings* of the Boston Society of Natural History.

At the period of the formation of the anlage of the permanent pharynx tissue-differentiation becomes active. Before considering this tissue-differentiation it may be best, however, first to go over

in brief outline the assumption by the embryo of the form characteristic of the adult.

In Fig. 1 are shown four stages in the development of the embryo. These figures were sketched by means of a camera lucida from living specimens. The outline of the intestinal cavity, as it appeared by transmitted light, is shown by serrated lines. In *D* the septa extending into the intestinal cavity are represented more highly developed than is common at this stage.

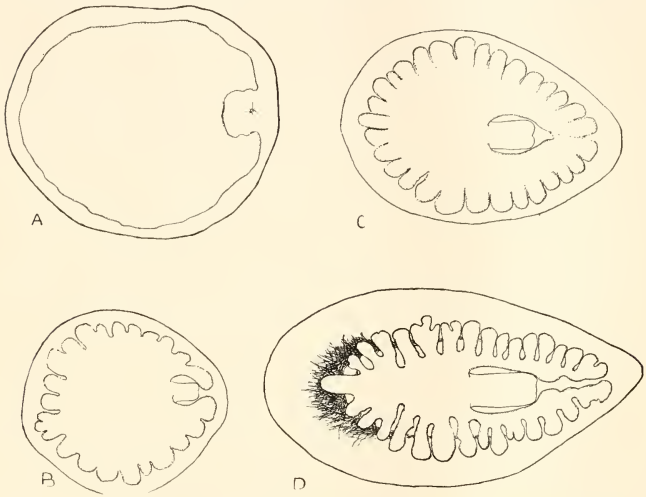


FIG. 1. Various stages in the development of the embryo. Magnification, 57 diameters.

A. Dorsal view of an embryo in which the anlage of the permanent pharynx is being developed. At the right the opening into the embryo-pharynx is shown.

B. Ventral view of an embryo in which the pharynx has begun to assume definite form.

C. Ventral view of an older embryo in which the two posterior extensions of the intestinal cavity have united behind the pharyngeal pocket.

D. Dorsal view of an embryo at the period when the central nervous system has assumed definite outlines.

This was done so as to expose the nervous system. The latter was reconstructed from sections made from the embryo. In *A* the opening into the embryo-pharynx is shown at the right. In

B the permanent pharynx is shown near the posterior end of the piece. This individual seems to have been a small one at the time of metamorphosis. In *C* and *D* the development of the tail posterior to the pharyngeal region is shown. There is a temporary anastomosis of the posterior rami behind the pharyngeal pocket. This anastomosis later disappears, as shown in Fig. 1, *D*. The head is meanwhile developed, as shown in the figures, anterior to the primitive yolk cavity.

The relations of the head, pharyneal and tail regions to the primitive yolk cavity of the embryo are also represented in Fig. 2. In *B* the anlage of the permanent pharynx is shown. The

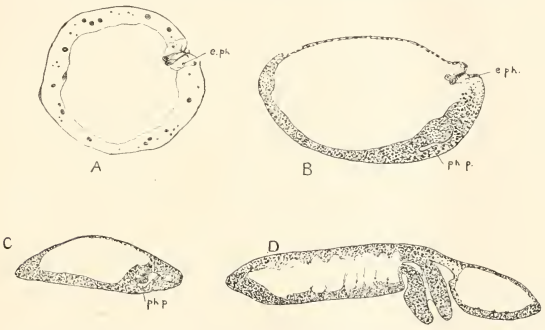


FIG. 2. Sagittal sections in the median line of embryos of various stages of development. Magnification, 57 diameters.

A. Section through an embryo which is becoming filled with yolk cells.

B. Section through an embryo in which both the embryo-pharynx and the anlage of the permanent pharynx are seen.

C. Section through an embryo in which the two posterior extensions of the intestinal cavity are uniting behind the pharyngeal pocket.

D. Section through an embryo in which the tail region is well developed and the anlage of the head is well marked. Owing to the longitudinal contraction caused by the killing fluid the pharynx has been partly forced out from the pharyngeal pocket. *e.ph.*, Embryo pharynx. *ph.p.*, Pharyngeal pocket.

base of the pharynx extends obliquely from the region of the embryo-pharynx to the ventral wall. In *C* the base of the pharynx still extends obliquely from the dorsal to the ventral walls. The embryo-pharynx has disappeared. A tail region posterior to the pharynx and a head region anterior to the yolk cavity are

distinctly developed. In *D* the assumption of adult condition is much more advanced. The base of the pharynx now extends nearly perpendicular from the dorsal to the ventral body wall. Head and tail regions are well developed.

The first indications of a definite nervous system appear in the region of the head at about the stage of development shown in Fig. 1, *C*. The outlines of the nervous system are, however, at this period too indefinite for satisfactory illustration. There are apparently bilateral anlages soon united by a commissure. The lateral cords gradually extend from the region of the brain towards the tail. At the period shown in Fig. 1, *D*, the central nervous system has the form there shown somewhat diagrammatically.

The median nerves and commissures and the lateral nerves probably arise by outgrowth from the central nervous system. Nothing is known of the development of the peripheral plexus.

Reproductive organs are developed only after the worm has advanced far in development. Curtis, (*op. cit.*) has followed the development of the reproductive organs in *Planaria maculata*. I shall therefore attempt no description of the process in this place.

Histogenesis in the Embryo.

The development of the various tissues during the formation of the structures characteristic of the adult is difficult to follow. For the sake of making a comparative study of the size and structure of the various cells at different stages of development I have used a uniform method of technique. The organisms were first anæsthetized with chloretone[1] to cause relaxation and to prevent the pre-mortem contraction which killing fluids are apt to stimulate. They were then fixed in a one-half saturated solution of corrosive sublimate to which acetic acid was added, carried through alcohol solutions of increasing strengths up to absolute alcohol, and thence into xylol. The tissues were embedded in paraffine, cut into sections $3\frac{1}{3}$ to $6\frac{2}{3}\,\mu$ thick, and stained in dilute Delafield's hæmatoxylin, followed by a solution of Congo red in water. A little Congo red was also added to the alcohol used in dehydration. The figures have been drawn to a fixed scale by means of the camera lucida.

[1] A term applied commercially to chloroform-acetone.

The surface epithelium is the simplest of the tissues to follow, owing to the sharp line of demarcation existing between it and the underlying tissues. Up to the time of the formation of the anlage of the permanent pharynx the surface membrane is beset but sparsely with cells. Fig. 3, *a*, shows one of these cells in cross-section. During the period of assumption of adult structure by the embryo these cells multiply rapidly by amitosis, as shown in Fig. 3, *b*. The cells increase in thickness, Fig. 3, *d*.

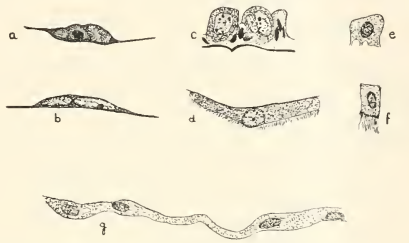


FIG. 3. Sections illustrating various stages in the development of the surface epithelium. Magnification, 540 diameters.

a. From the embryo shown in Fig. 1, *A*.

b. From an embryo in which the pharyngeal anlage is being formed.

c. Dorsal epithelium near the pharyngeal region of an embryo in which the nervous system is beginning to appear.

d. Epithelium from the ventral surface of the tail region of the same embryo.

e. Dorsal epithelial cells from an adult individual.

f. Ventral epithelial cell from an adult individual.

g. Epithelium covering a cut surface of an adult individual twelve hours after injury.

In *b* and *c* evidences of direct nuclear division may be seen.

Finally the membrane becomes covered externally with cells so crowded together that the long axis of each cell lies at right angles to the surface of the body, Fig. 3, *c*. This crowding first takes place near the center of the dorsal and ventral surfaces, where bodily extension is least rapid, and only later at the growing extremities of the worm (compare *c* and *d*, Fig. 3). The dorsal epithelium at no time is ciliated (Fig. 3, *c*), the ventral epithelium acquires cilia at an early stage of development (Fig. 3, *d*). Although it is commonly stated in the text-books that fresh-water planarians are completely covered with cilia when

young, I have seen no specimens of *Planaria maculata* at any stage of development in which the dorsal epithelium was ciliated. For the sake of comparison a dorsal and a ventral epithelial cell from an adult individual are shown in *e* and *f* of Fig. 3. These seem to be slightly smaller than the embryo cells. Although the possibility cannot be excluded that cells are added to the surface epithelium from the parenchyma, I have found no evidence of this.

The cells of the parenchyma offer a most difficult and uncertain subject for study. Cells of the type shown in Fig. 4, *c*, are

FIG. 4. Sections illustrating the development of the parenchyma. Magnification, 540 diameters.

a. One of the cells surrounding the yolk cavity of the embryo shown in Fig. 1, *A*.

b. Corresponding cell of a slightly older embryo.

c. Type of cell prevailing in the parenchyma of an embryo immediately before the stage when the pharyngeal anlage appears.

d. Similar cell showing evidence of amœboid movement.

e. Cells illustrating the reduction in size which takes place during the tissue differentiation that occurs at the time when the pharyngeal anlage appears.

f. Closely packed cells seen in the anlage of the permanent pharynx.

g. Connective-tissue cells of body wall during this stage.

h. Early rhabdite cell.

i. Connective-tissue cells of adult individual.

j. Large parenchymal cell in resting stage.

k, *l*. Large parenchymal cells undergoing division.

m. Cells from anlage of head thirty-eight hours after isolation of the piece.

those most commonly met with at the stage preceding the development of the permanent pharynx. In certain regions, such as the pharyngeal anlage, these cells may be very closely packed together (Fig. 4, *f*), but if we assume them to form a syncytium we must consider it a syncytium in which the cell territories are fairly well marked. The individual cells may be irregular in shape or distinctly elongated. From cells of this character

smaller cells with smaller nuclei arise and form a syncytium of branched cells (Fig. 4, *g*). The connective tissue of the adult is derived from cells of this nature (Fig. 4, *i*), fibrils in the processes connecting the cells becoming ever more distinct. In addition to these fixed connective-tissue cells there exist in the parenchyma of the adult several other types of cells. Some of these, with dark protoplasm, large nuclei, and well-marked nucleoli lying in clear areas, seem to be the direct, undifferentiated descendants of the embryonic cells of the type shown in Fig. 4, *e*. These cells divide by mitosis (Fig. 4, *k* and *l*) and seem to be the chief factor in the regeneration of new parenchyma. Other cells with lighter nuclei and protoplasm the granules of which stain deeply in "acid" dyes may be leucocytes, as Miss Stevens has aptly suggested.[1] Cells of this nature are seen at an early stage and apparently are derived from cells of the type shown in Fig. 4, *e*. Rhabdite cells begin to be differentiated even before the period when the epithelium assumes a columnar structure, but while this latter process is taking place the rhabdite cells approach the basement-membrane and rhabdites are passed out into the intercellular areas. In Fig. 3, *c*, the depression in the membrane indicates the area through which rhabdites have been passed. I have seen no clear indications of the passage of the rhabdite cells themselves through the membrane. Mucus cells and the gland cells belonging to the intestinal system arise at a comparatively late stage. I have not been able to trace their origin from any specific cells.

When lining surfaces like the pharyngeal pocket the parenchyma cells assume an epithelial character. These cells have an origin similar to that of the connective-tissue cells.

Intestinal cells become clearly evident at the time when the embryo assumes an ovoid form. They are at first sparingly scattered, but at the time when septa extend into the yolk-cavity so as to give rise to the permanent form of the intestinal system, they begin to multiply by indirect division (Fig. 5, *a*). As in the case of the surface epithelium, this cell multiplication finally gives rise to cells extending away from the base on which they rest

[1] "Notes on Regeneration in *Planaria lugubris*," *Archiv f. Entwickelungsmechanik der Organismen*, XIII., p. 410, 1901.

(Fig. 5, *b*). As pointed out by Iijima, however, this last process is slow, and it is only at a comparatively late period in development that the intestinal epithelium assumes the structure characteristic of the adult. Whether the intestinal epithelium is differentiated at a very early period and then preserves its specificity, like the surface epithelium, or is added to by constant additions from the parenchyma, it is difficult to decide. The former view seems to me perhaps more probable. It must be stated in

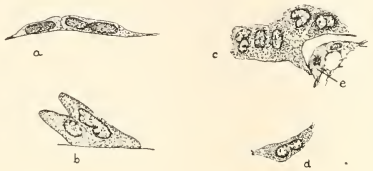


FIG. 5. Sections illustrating the development of the intestinal epithelium. Magnification, 540 diameters.

a. Flat cells seen in embryos at the time of the development of the permanent pharynx.

b. Cells beginning to project toward the lumen of the intestine in an embryo in which the nervous system has just become clearly visible.

c, *d*. Intestinal cells undergoing direct division during regeneration in the adult.

e. Cells bordering on the intestines. In one cell a mitotic figure is visible.

this connection, however, that the parenchyma cells bordering on the intestines show active proliferation by mitosis during embryonic development as well as during regeneration (see Fig. 5, *c*).

Myoblasts become numerous at about the period of the formation of the anlage of the permanent pharynx (Fig. 6, *a*). They are very large cells and apparently are derived from cells of the type shown in Fig. 4, *c*, many of which have more vesicular nuclei than there shown. The myoblasts seem to divide by indirect division. From those lying next the basement membrane of the epithelium is derived a syncytium, a portion of which is shown in Fig. 6, *b*. In this the surface musculature is developed. It is probable that a separate syncytial sheet exists for each layer of musculature. Other myoblasts give rise to the muscle fibers which run through the parenchyma in various directions.

Blochmann,[1] who studied the tissues of cestodes by means of the methylen-blue and silver impregnation methods, describes as "Sommer-Landois cells" large cells lying below the surface musculature and sending out branches connected on the one hand with the fibers of the surface musculature and on the other hand extending into the peripheral nerve plexus. The muscle fibers he

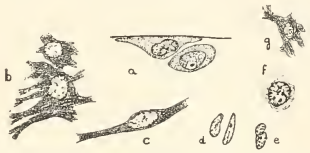


FIG. 6. Sections illustrating the development of the musculature. Magnification, 540 diameters.

a. Myoblasts in an embryo in which the pharyngeal anlage is being formed.
b. Developing circular musculature at a later stage.
c. Developing parenchymal muscle cell.
d, *e*. Small nuclei lying between the muscle fibers of an adult individual.
f. Large cell occupying a corresponding position.
g. Developing circular musculature during regeneration.

considers outgrowths of processes of these cells. In *Planaria maculata* large cells with vesicular nuclei, resembling in size and form the myoblasts of the embryo, may be seen lying below the surface musculature. Whether or not these represent Sommer-Landois cells which have migrated from the syncytial musculature after the formation of the latter cannot be decided by methods which I have employed. Monti has described cells of the Sommer-Landois type in planarians.[2]

It is uncertain whether the musculature of the pharynx arises from the cells potentially differentiated at the time of the formation of the pharyngeal anlage.

Of all the tissues the nervous tissues are the most difficult to follow in these animals. Only by special technical methods can this be satisfactorily done and yet the planarian tissues seem very refractory to the ordinary impregnation methods. Blochmann (*op. cit.*) used these successfully on cestodes and gives an inter-

[1] "Ueber freie Nervenendigungen und Sinneszellen der Bandwürmern," *Biologisches Centralblatt*, XV., 14, 1895.

[2] *Archives Italiennes de Biologie*, Vol. XXVII., p. 15, 1897.

esting description of the elements of the peripheral nerve plexus. In this many nerve cells are found. Monti[1] has stained by the Golgi method and described unipolar, bipolar and multipolar cells lying both in and about the brain and nerve cords and in the periphery next the musculature but she has not followed their development. Bergendal[2] found in the central nervous system of land planarians large ganglion cells, with very large nuclei which stain lightly.

I have made no extended attempt to stain the nervous system of planarians by special methods. In and about the nerve-cords and brain are scattered nuclei that stain very lightly and are much larger than the nuclei of the fixed connective-tissue cells (see Fig. 7, *e*, and compare with Fig. 4, *i*). These I have taken to be the nuclei of nerve cells. The cell-body is usually difficult to follow, but at times this may be seen sending off one or more

FIG. 7. Sections illustrating the development of the nervous system. Magnification, 540 diameters.

a. Large cell seen in the anterior ventral region of an embryo before the anlage of the pharynx has appeared.

b. Similar cells after the anlage has appeared.

c. Early stage in the development of nerve fibers.

d. Nerve cells in an adult individual.

e. Row of nerve cells seen in a regenerating nerve cord.

f. Nuclei of nerve cells undergoing direct division during regeneration of a new brain.

processes. At the stage of development in the embryo immediately preceding the formation of the permanent pharynx a few large, palely staining cells may be seen in the anterior portion of the ventral body wall. A cell of this kind is shown in Fig. 7, *a*. I have not found these cells elsewhere in these embryos and think it possible that they represent neuroblasts. I have tried to find them in earlier stages of development without success. They

[1] "Sur le system nerveux des Dendroceles d'eau douce," *Archiv. Ital. de Biologie*, Vol. XXVII., 15, 1897.

[2] *Zoölogischer Anzeiger*, X., 218–224, 1887.

do not seem at this period to arise from the surface epithelium. At the time of the formation of the anlage of the permanent pharynx, cells like these in a corresponding region may be seen multiplying by direct division (Fig. 7, *b*). When the brain anlages first become clear, owing to the formation of nerve fibers, similar cells may be seen lying among the fibers (Fig. 7, *c*). In the nerve cords, as they grow toward the tail region, corresponding nuclei may be seen, often in columns and often appearing to divide by direct division. It seems possible, therefore, that the nerve cells of the central nerve system of these planarians may arise by direct division from neuroblasts which are differentiated in the anterior ventral wall of the embryo at a comparatively early period, and that the other cells of the central nervous system may arise by direct division from these.

The cells of the peripheral nerve plexus may arise, however, in the periphery and not from those of the central nervous system. In the adult many very pale nuclei may be seen scattered here and there below the musculature. These nuclei resemble those of the cells found within the central nervous system.

During embryonic development, therefore, we find that the surface epithelial cells are derived by direct division from the pre-existing ectoderm cells. The intestinal cells certainly, and also probably the nerve cells, multiply by indirect division. Whether additions are made from parenchyma cells after the primary period of differentiation is uncertain. The early myoblasts multiply partly, at least, by indirect division. The period at which the differentiation of myoblasts from the parenchyma ceases is undetermined. Branched, anastomosing connective-tissue cells, "leucocytes," rhabdite cells and gland cells are differentiated in the parenchyma but certain cells retain a size and form characteristic of an early stage of embryonic development.

Regeneration in Embryos.

When removed from the egg-capsule embryos of the stages represented in Fig. 1, *A* and *B*, and younger ones live but a short time in water. Twenty-four hours is the longest I have been able to keep them alive. Embryos of the stage shown in Fig.

1, *C*, may, with care, be kept alive until adult form is assumed; but if mechanically injured they do not long survive and pieces removed from them will not live. If embryos of the stage shown in Fig. 1, *D*, be cut in a region immediately anterior to the pharynx both anterior and posterior pieces may sometimes be kept alive. The anterior piece will develop a new pharynx, but the posterior piece will not develop a new head, although it may otherwise undergo full development, including the assumption of the pigmentation characteristic of the adult. It seems probable, although it cannot be taken as proved, that the failure of the posterior piece to give rise to a new head is due to the absence in the piece of well-formed nerve cords. If the cut be made near the head or in older embryos a new head may be regenerated.

In *Dendrocœlum lacteum*[1] F. R. Lillie found that a new tail may be formed by outgrowth from any transverse level behind the region immediately back of the eyes, but a head only on a cut surface made in the anterior third of the body. These results have been confirmed by E. Schultze[2] and by me. Lillie ascribes the result to the cephalization of the nervous system in *D. lacteum*. It is interesting to note that at the period when the central nervous system is "cephalized" in *Planaria maculata* similar conditions prevail.

Regenerative Development.

For the sake of comparing embryonic with regenerative development we may consider briefly the regeneration of new individuals from cross pieces taken from in front of the pharynx, from tail pieces and from slips removed from a region lateral to the median line.

Cross Pieces.—There is a close similarity between the development of a new individual from a cross piece cut from in front of the pharynx and development in the embryo. In the cross piece the pharynx develops on the posterior ventral surface, with relations to the axial gut similar to that borne by the pharynx to the yolk cavity in the embryo (see Fig. 8). The head and tail re-

[1] "Notes on Regeneration and Regulation in Planarians," *American Journal of Physiology*, Vol. VI., p. 128, 1901.

[2] "Aus dem Gebiete der Regeneration bei Turbellarien," *Zeitschrift für wissenschaftliche Zoölogie*, Vol. LXXII., p. 1, 1902.

gions are likewise developed in a manner resembling the embryonic.

If the cut surface on which the new head is developed be at right angles to the long axis of the parent worm the new head will be developed symmetrically about the anterior extremity of the axial gut, just as it is developed symmetrically about the anterior extremity of the yolk-cavity in the embryo. If, however, the cut be made oblique, the head may arise some distance anterior and lateral to the region where the old axial gut projects on the cut surface. Morgan first described this phenomenon.

FIG. 8. To illustrate the development of a new individual from a cross piece 48 hours after removal. Magnification, 57 diameters.

A. Individual as seen from the dorsal surface. The intestinal system was sketched from the living individual, the nervous system was reconstructed from sections.

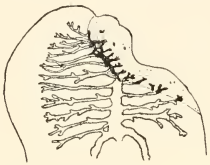
B. Sagittal section in the median line from the same individual. *ph.p.*, Pharyngeal pocket.

Fig. 9 shows a good example from *Dendrocœlum lacteum*. The median ends of the lateral intestinal branches lying in the piece anterior to the extremity of the old axial gut and separated from the latter by the cut have become so anastomosed as to form an extension of the old axial gut. About the anterior extremity of this the new head is being symmetrically differentiated. In the further growth of the piece new tissue is rapidly formed to the right of the axial gut and about its extremity until the worm is restored to normal proportions.

Tail Pieces. — In tail pieces regeneration of the new head takes place symmetrically about a branch extending forward from the area of fusion of the two posterior rami of the original piece. In all of the instances which I have examined this fusion has taken place before differentiation of a head has really begun. The process of head formation resembles essentially that described as taking place on the anterior surface of the cross piece taken from in front of the pharyngeal region. The formation of the pharynx

is the most puzzling process in the regeneration of these tail pieces.

The region of pharynx formation depends upon the condition of the piece and the region of the cut. In individuals not sexually mature if the cut be made posterior to the old pharynx the new pharynx will develop slightly anterior to the center of the piece (Fig. 11, *B*). I have followed the development of a large number of such specimens during a cool spell when the regenerative processes were somewhat slow, and the successive stages in regeneration therefore well marked. The following notes in-



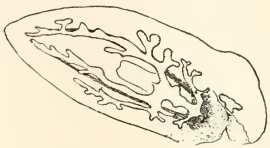


FIG. 9. To illustrate the formation of a new axial gut lateral to the axis of the original gut, after oblique section; *D. lacteum*. Magnification, 17 diameters. The regenerated intestinal branches are shown in black.

FIG. 10. To illustrate the development of a head symmetrically about the end of the axial gut and posterior to an anterior lateral slip which contained no nervous system. Magnification, 17 diameters.

dicate these stages. The number of hours given in each instance shows the length of time elapsing after isolation of the pieces:

Eighteen hours. The cut surface is covered with epithelium and there is some accumulation of parenchyma cells near it.

Thirty-eight hours. Further accumulation of cells near the cut surface.

Sixty-six hours. Anastomosis of the posterior intestinal rami in the vicinity of the cut surface (Fig. 11, *A*). Advance of nerve fibers into the head anlage. In some specimens there is a slight accumulation of cells in the vicinity of the future pharynx.

Ninety hours. The accumulation of cells in the pharyngeal anlage is more marked. Between the region of anastomosis of the posterior rami and the pharyngeal anlage new intestinal branches are being formed. The head anlage has advanced in development.

One hundred and fourteen hours. Considerable anastomosis

between the intestinal branches last mentioned. In some specimens a new axial intestine had thus been formed between the anlage of the head and that of the pharynx (Fig. 11, *B*). The pharynx is beginning to be differentiated. The head is much advanced in development but the chief commissure of the brain is not completely formed.

One hundred and thirty-eight hours. The axial intestine and pharynx are well formed. The chief commissure of the brain is well formed and the eyes have appeared.

Subsequently the axial intestine formed between the two original posterior rami becomes greatly developed at the expense of the latter.

Sometimes the process is more simple than that described above, the anterior portion of one or the other of the posterior rami becoming converted directly into an axial gut; or fusion of two rami may be more complete, as in the instance described in my previous paper.[1] Miss Stevens (*op. cit.*) states that the accumulation of cells for the pharyngeal anlage may begin in *P. lugubris* before intestinal anastomosis takes place. We must admit, therefore, that in instances of this kind the pharyngeal anlage is not formed in response to stimuli arising from a definite axial intestinal cavity, as seems to be the case in the embryo and in pieces cut anterior to the pharynx, but rather upon the relations borne by a given area of the worm to the intestinal system as a whole.

In sexually mature worms cut immediately anterior to the genital apparatus the pharynx is formed much further anterior than in sexually immature specimens. The reproductive organs undergo retrograde metamorphosis. Destruction of the genitals begins at the periphery of these organs where the intestines border upon them and extends towards the center (see Fig. 11, *C*). The process is a complex one which I shall not attempt to describe in this place. The ducts and glands, including the testicles, also become disorganized, the last to disappear being those near the posterior end of the animal. As a result of the breaking down of these organs the neighboring tissues of the planarian

[1] "On the Physiology of Regeneration in *Planaria maculata* with Especial Reference to the Phenomena of Regeneration," *American Journal of Physiology*, Vol. V., p. 1, 1901.

become filled with bits of disorganized tissue. Most of this eventually finds its way into the intestines which become thereby so distended that in many places the lining epithelium is as thin as that found during embryonic development. Stevens (*op. cit.*) has described a similar process in *P. lugubris.* The pharyngeal anlage is formed in the median line immediately anterior to the degenerating sexual apparatus and posterior to the center of the anastomosis between the posterior rami (Fig. 11, *c*).

If the cut isolating the tail piece pass through the pharyngeal pocket the new pharynx will develop at the anterior end of the

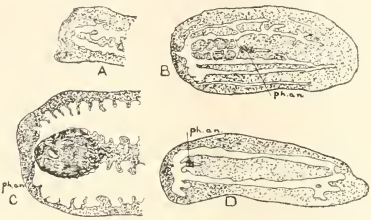


FIG. 11. To illustrate the development of new individuals from tail pieces. Magnification, 17 diameters.

A. Intestinal anastomosis, sixty-six hours after isolation of the piece.

B. Intestinal anastomosis ninety hours after isolation of the piece. The pharyngeal anlage is indicated, *ph.an.*

A and *B* are from individuals not sexually mature.

C. Piece from an individual sexually mature. The reproductive organs are undergoing degeneration.

D. Tail piece isolated by a section passing through the pharyngeal pocket.

pocket. If a portion of the tip of the old pharynx be retained in the chamber this tip will be utilized to form the end of the new pharynx.[1]

Lateral Slips.—In lateral slips isolated by a cut lateral to the region of a lateral nerve cord no specific regeneration takes place. This, at least, is the result I have obtained not only in *P. maculata* but also in *P. lugubris*, *Dendrocalum lacteum* and *Phagocata gracilis.* If a part of the lateral cord be present in such a

[1] The process of retrograde metamorphosis of the retained portion of the pharynx which I previously described (*op. cit.*, p. 34) is the exception, not the rule. If a portion either of the base or the tip of the pharynx be retained in the piece it will usually be utilized in the regeneration of the new pharynx.

slip a new head will be regenerated, and if a fairly considerable amount of the intestinal apparatus be contained in the slip, a new pharynx will be formed. The intestinal apparatus, if it originally consists of isolated parts, tends to become anastomosed into a whole. A new head is formed about that portion of the intestinal system which projects on the cut surface in the neighborhood of the most developed portion of the nerve-cord (see Fig. 12, *A*). The new pharynx is formed posterior to the region of least bodily compression, polarity being understood to be determined by the nervous system. If the lateral slip be cut so that

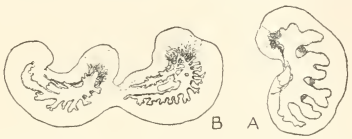


FIG. 12. To illustrate the development of an individual from a lateral slip. Magnification, 17 diameters.

A. Piece isolated from the region lateral to the pharyngeal pocket.

B. Two individuals developing from a lateral slip from the center of which a portion of the nerve cord was removed.

two or more regions will be formed in which the nervous and intestinal apparatus of each section will be isolated from that of the neighboring section, double or even triple individuals may be formed. Fig 12, *B*, indicates a double individual of this kind.

Having thus briefly considered the regeneration of new individuals from these typical isolated pieces, we may now take up, first, the question of histogenesis during regeneration and, second, that of the conditions determining the formation of definite structures.

HISTOGENESIS DURING REGENERATION.

After removal of the piece the surface epithelium spreads out so as to cover the cut area with a layer of epithelium like that covering the embryo, but somewhat thicker than the latter (see Fig. 3, *g*). Stevens (*op. cit.*) gives an excellent account of this process in *Planaria lugubris*. Direct cell division restores the epithelium to normal form. The cells regenerated on the dorsal surface are not ciliated.

The most active cells in the development of the parenchyma of the new part are cells which closely resemble the cells playing a similar part in embryonic development (Fig. 4, *c*, *k*, *l*) and, like them, they divide by indirect division. These cells probably represent the "Stammzellen" mentioned by Keller.[1] The many branched connective-tissue cells seem also to assume a spherical form and to multiply by indirect division. The new connective tissue (Fig. 4, *m*) resembles the embryonic (Fig. 4, *f*). The new surface musculature may arise from the large cells shown in Fig. 6, *f*, cells which resemble the embryo myoblasts. The musculature of the parenchyma seems, however, to arise from cells of the type shown in Fig. 4, *h* and *l*, as do also the rhabdite cells.

The epithelium lining the intestinal branches which extend into the new parts apparently multiplies by direct division (Fig. 5, *c* and *d*).

Cells bordering immediately upon the intestines multiply by indirect division (Fig. 5, *e*). I am uncertain whether these cells contribute to the supply of the intestinal epithelium, but am inclined to think that they do not. Stevens (*op. cit.*) thinks that they may. The new nervous system arises by direct extension of fibers and apparently also of cells from the old into the new parts. Fig. 7, *c*, represents a growing bundle of cells and fibers of this nature. Fig. 7, *f*, shows direct nuclear division in the vesicular nuclei of the cells which accompany the fibers into the new parts. At present, however, no certain evidence can be offered that parenchyma cells do not also become directly transformed into nerve cells under the influence of the old nerve cords. The majority of investigators believe that the nervous system is differentiated from the parenchyma, though in direct continuity with the old nervous system.[2]

The reproductive organs seem to arise from the cells of the type shown in Fig. 4, *c*. During their development mitosis is

[1] "Die ungeschlechtliche Fortpflanzung der Süsswasser Turbellarien, *Jenaische Zeitschrift f. Naturwissenschaften*, Vol. XXI, p. 370, 1894.

[2] Flexner, *Journal of Morphology*, Vol. XIV., p. 337, 1898. Stevens, *op. cit.* Morgan, "Growth and Regeneration in *Planaria lugubris*, *Archiv für Entwickelungsmechanik*," Vol. XIII., p. 179, 1901. E. Schultz, "Aus dem Gebiete der Regeneration bei Turbellarien," *Zeitschrift für wissenschaftliche Zoölogie*, Vol. LXXII., p. 1, 1902.

active. E. Schultz (*op. cit.*) has given an excellent account of the regeneration of the reproductive organs in *D. lacteum*. Morgan has shown (*op. cit.*) that in *P. lugubris* pieces cut anterior to the old reproductive organs may regenerate them.

An exceptionally good discussion of histogenesis in forms allied to the planarians may be found in the article by Keller (*op. cit.*).

Factors Determining Development.

Having thus considered the origin of the tissues entering into the new parts, we may now consider the factors concerned in the development of the various regions in planarians. Of these regions the most definite and fundamental are the head and pharynx. In the embryo the head is developed at one extremity of the yolk-cavity, the pharynx at the other. Between these two regions the yolk-cavity is converted by the ingrowth of septa into an axial gut with lateral branches. The axial gut grows forward into the developing head and posteriorly it sends two branches into the caudal region which develops behind the pharynx.

In regeneration we likewise find that pharynx and head are developed with certain relations to the intestinal system of the isolated piece. Brought near to one another by muscular contraction isolated intestinal branches become anastomosed into a unit system. By making certain cuts it is possible to have two or more such unit systems developed in a single piece, as shown in Fig. 12, *B*. Head and pharynx are developed in connection with such a unit system only when there is contained within the piece a portion of the central nervous system. This is shown in Fig. 10, where the unit intestinal system of the anterior lateral slip has neither head nor pharynx developed in connection with it. Furthermore, the piece containing the unit system must be above a minimum size. Thus in very short head pieces and short, narrow lateral slips no pharynx may be developed although the piece contain a portion of the original intestinal system and a portion of the original nervous system.

What determines the location of the anlage of the pharynx? In a previous paper (*op. cit.*) I showed that the pharynx arises in a region posterior to that portion of the intestinal system into

which, on general bodily contraction, the contents of the whole intestinal system tend to be forced. I called this portion of the intestinal system the area of "least intestinal pressure" but this term is bad because it admits of several interpretations. The area into which the intestinal contents tend, on general contraction, to be forced, and in which the new pharynx arises, is essentially the area of least bodily compression. In *P. maculata* the stimulus to pharyngeal formation is exerted in an antero-posterior direction in this region. Polarity in the piece is determined by the central nervous system. The pharyngeal anlage first appears near the ventral wall of the body.

In the embryo it seems fair to assume that the contents of the yolk-cavity are forced toward the embryo-pharynx on general contraction. Closure of this would cause a stimulus to pharynx formation to be exerted on the body wall comparable to that exerted in regenerative development.

In cross pieces taken from in front of the pharynx and in lateral slips the relation of pharynx to intestines is fairly clear (Fig. 8 and Fig. 11).

In tail pieces the problem is more complicated. When no reproductive organs are present the pressure exerted within the anterior extremities of the posterior intestinal rami by contraction of the piece tends to force the intestinal contents into the anterior median branches of the posterior rami, and thus to give rise to an intestinal area not at first occupied by a single intestinal cavity, or axial gut, but by several distended branches. Posterior to this area pharyngeal formation may be started even before the median branches of the posterior rami have fused to form an axial gut. When, however, a portion of the pharyngeal pocket or the sexual organs are contained in the piece the anterior median branches of the posterior rami are thereby prevented from expanding so that the intestinal contents are forced further forward into the area anterior to the pharyngeal pocket or to the reproductive organs.

The exact nature of the stimulus giving rise to pharyngeal formation is still in the dark, although we can point out the area where it seems to be exerted. It is fairly certain, however, that bodily fluids are forced in considerable quantity into the area of the pharyngeal anlage.

In *Phagocata gracilis* F. R. Lillie[1] has shown that in the pharyngeal anlage a considerable number of pharyngeal pouches are developed and that these subsequently fuse. In lateral slips the pharyngeal pouches on the cut side are formed only subsequently to the development of an intestinal branch on that side. In this animal the pharyngeal area seems to be determined by factors similar in nature to those at play in *Planaria maculata*, but pharyngeal formation in this area is limited not to a single stimulus exerted posteriorly from the region of the axial gut, but, on the contrary, may be excited by stimuli arising from anterior median branches of the chief posterior rami of the developing intestinal system. For a criticism of the view which I have expressed as to the factors determining the seat of pharyngeal formation the reader is referred to Lillie's article.

The pharynx may be formed either near a cut surface or quite within the body of the piece. A new head is formed only in the tissue produced on a cut surface. This surface may be a flat plane. In such a case the median axis of the head will at first be perpendicular to the cut surface. Or the head may be produced at the junction of two planes. Thus, if the anterior end of the worm be removed by a V-shaped cut, the angle of the V pointing posteriorly, and the two sides be allowed to unite, as a rule a new head will be produced, the median axis of which is in line with the chief axis of the worm. If they be not allowed to unite a head will be produced on each side with its median axis perpendicular to the plane of the cut. If the V point anteriorly sometimes a head will be produced on each cut surface; at other times a single head will arise, the median axis of which corresponds with the longitudinal axis of the worm. When a new head arises thus at the juncture of two plane surfaces the median axis of the head seems to bisect the angle formed by the junction of the two cut surfaces. Of course, the cut surfaces may not be planes, but may be very irregular in shape. In all such instances, however, similar axial relations prevail.

The direct stimulus to head and brain formation seems to be due to the presence near the cut surface of the chief coördi-

[1] "Notes on Regeneration and Regulation in Planarians, *American Journal of Physiology*, Vol. VI., p. 134, 1901."

nating area of the nervous system of the piece. In most cases of regeneration of a new head, this means the most anterior portion of the old central nervous system. Lillie has stated this point of view very clearly in the article referred to above. But while the stimulus to brain formation arises from the chief coördinating center of the piece, the head and the brain are symmetrically formed about a center of nutrition which is represented by that portion of the intestinal system which lies immediately adjacent to the nerve center, and which is in most direct continuity with the main intestinal apparatus. A symmetrical head is formed both when the stimulus to brain formation comes from one side and when it comes from both sides of this projecting intestinal tip.

There is an interesting correlation between the lateral nerve cords and the eyes. In oblique pieces the most direct stimulus to brain formation comes from that nerve cord which is the more anteriorly situated. As a rule the eye on the side corresponding to this nerve cord is developed earlier, and at first larger, than that upon the other. This is still more marked in the regeneration of oblique cross pieces divided in the median line, as shown by Morgan (*op. cit.* 1901). So, too, in the regeneration of lateral slips the eye on the outer side is usually earlier developed than that on the median.

That stimulus to brain formation depends upon the central nervous system projecting into the area of the cut and that the head is formed symmetrically about the tip of the axial gut, with the cut surface as a base, is indicated by the following experiment. If a longitudinal cut be made lateral to the nerve cord from the region of the head back nearly to the pharynx, and then transversely across the body, the slip thus left attached to the main piece will contain none of the central nervous system of the animal. The slip will contract posteriorly and will bend over so as to become fused to the transverse cut surface. When regeneration is well under way it will be found that the new head is developing on the transverse cut surface, symmetrically about the tip of the old axial gut, as shown in Fig. 10. The contracted anterior slip exhibits no specific influence on the regeneration of the head, although the latter is continuous with

it. Subsequently, normal form is usually restored by forward growth of the head region and absorption of the lateral slip. By making two such anterior lateral slips, one on each side, it is possible to get a head to develop posterior to a ring of tissue formed by the anastomosis of these slips. As the head grows it projects dorsally in a forward direction beyond the anastomosing lateral slips.

The influence exerted by the intestinal apparatus upon the regenerating part is probably in the main a nutritive one. Owing to the action of the musculature upon the intestinal system that region of the periphery of the body which receives the richest nutritive supply is probably the area about the tip of the axial gut where the head is differentiated. Owing to its proximity to the axial gut the whole anterior regenerating region also receives a rich nutritive supply and this may account for its rapid growth until normal proportions are reached.

The effect of proximity to the axial gut on regenerating tissue is illustrated by the following experiment.

If a narrow longitudinal slip of tissue be removed from one side of the anterior half of the body and a broad slip from the other, regeneration of tissues will be the more rapid on that side from which the broad slip has been removed. This area of more rapid regeneration lies nearer the axial gut.

Morgan (*op. cit.*, p. 200) gives a picture of an oblique cross piece in which the anlage of a new head has appeared before intestinal anastomosis is visible between the pharyngeal area and the base of the head. Morgan traced the outlines of the intestinal system of the piece from the pigment particles contained within the intestinal cells. I have previously shown, however (*op. cit.*, p. 22), that but little pigment is found in the newly formed anastomotic intestinal branches, so that in the piece mentioned by Morgan an anastomotic axial gut may have existed. By using small, slightly pigmented, starved worms in which the reproductive organs are not developed the intestinal outlines may be fairly accurately followed during life, if the pressure of a cover-glass be used to flatten the specimens. Oblique illumination is often of aid. In the hundreds of generating pieces of planarian which I have examined, I have never seen an instance in which

differentiation of a head has taken place otherwise than about the tip of an intestinal branch connected with the main intestinal apparatus of the animal. This intestinal branch may be termed the axial gut because of its subsequent relations to the regenerated worm. Lillie, in a valuable contribution to the subject of regeneration in planarians (*op. cit.*, p. 141), states that "the intestinal system regenerates in relation to the new external parts and not *vice versa*, as maintained by Bardeen; from which it follows that the location of the new parts cannot be due primarily to the form of the gut." As a matter of fact it is probable that mutual relations exist between the intestinal system of the animal and the regenerating parts, each exerting specific influences upon the growth of the other.

After the head and pharynx have been formed, the processes which serve to restore the worm to normal proportions become very marked and, unless certain conditions prevail, such as lack of size or attachment to another individuality (as shown in Fig. 12, *B*), this is soon effected. Bilateral symmetry, however, may arise in partially detached pieces containing neither head nor pharynx. Morgan has made a special and valuable study of the general gross relations borne by an isolated part to the regenerated individual.[1]

In small pieces isolated from sexually mature worms the reproductive organs at first completely disappear, as shown above in discussing regeneration in tail pieces. They are regenerated only after the worm has reached a certain advanced stage of development. In *Planaria maculata*, furthermore, the development of the sexual organs seems to depend upon season. In *Dendrocœlum lacteum* Iijima found that after depositing the cocoons the individuals die, but this is not true of specimens of *P. maculata* kept in captivity. It is probable that there is great variation in different species of planarians in the time of development and the stability of the reproductive organs. Schultze, as mentioned above, gives an excellent account of the regeneration of the reproductive organs in *D. lacteum* (*op. cit.*).

[1] *Op. cit.*, and also "The Internal Influences that Determine the Relative Size of Double Structures in *Planaria lugubris*," BIOLOGICAL BULLETIN, Vol. III., p. 132, 1902.

The destruction of the genital apparatus in isolated tail pieces shows that in the reorganization which takes place during regenerative development highly differentiated tissues, like the musculature of the genitals, are not transformed into new parts, but instead undergo retrograde metamorphosis and serve probably as food stuff for the other tissues. On the other hand, highly organized parts may be utilized directly in the formation of new organs. This is shown by the union of a tip of the pharynx retained in the pharyngeal pocket to a regenerated base.

Summary.

1. The anlage of the pharynx is formed at one extremity of the yolk-cavity of the embryo, that of the head at the other.

2. Up to the period of the formation of the pharynx the yolk-cavity is surrounded by a layer of parenchymal cells and these by a thin epithelial sheet. The parenchymal cells multiply by direct and by indirect division and become reduced in size as they increase in number.

3. The ectodermal cells begin to multiply rapidly by direct division after the pharynx has been formed, and thus give rise to a columnar epithelium. The dorsal cells at no time develop cilia, while the ventral cells produce them before becoming columnar in form.

4. Tissue differentiation begins in the parenchyma shortly before the pharyngeal anlage appears. From the parenchyma are developed intestinal cells, muscle cells, nerve cells, rhabdite cells and gland cells, as well as branched connective-tissue cells and possibly leucocytes. Certain of the parenchyma cells seem to multiply without undergoing further differentiation. Intestinal, nerve and muscle cells multiply by direct division, while the parenchyma cells multiply mainly by indirect division.

5. The central nervous system is first developed in the region of the head and from there the lateral cords grow posteriorly.

6. When removed from the cocoon very young embryos soon die. Slightly older ones die if injured. After the pharynx has become functional the embryos may be cut into two or more parts which will live. Pieces from which the head has been removed will not develop a new head unless they contain well-developed nerve cords.

7. In regeneration the new ectodermal cells arise from the old by direct division. New nerve cells, intestinal cells and muscle cells seem likewise to arise from preëxisting cells by direct division. It is possible, however, that they may spring from the large cells of embryonic type situated in the parenchyma. From the latter the other tissues mainly arise.

8. The new pharynx arises immediately posterior and ventral to the region into which the intestinal contents are forced by general muscular contraction and in response to stimuli arising from this region.

9. A new head is regenerated only in tissue produced at a cut surface, and with definite axial relations to the surface. The direct stimulus to head formation arises from an exposed chief coördinating region of the central nervous system. The head is formed with radial symmetry about the tip of the main intestinal branch extending to the cut surface in the vicinity of the exposed nervous system.

10. After head and pharynx have been differentiated the activities tending to restore the piece to a worm of normal form and proportions become especially marked.

11. During regeneration highly differentiated tissues are destroyed unless they may be directly utilized in the formation of new parts.

A CASE OF ABNORMAL PLUMAGE.

R. M. STRONG.

The purpose of this paper is to describe a case of abnormal plumage. The material was obtained from a hybrid pigeon belonging to Professor C. O. Whitman, which was reared at Woods Holl, Mass., in the summer of 1902. The male parent of the bird was a ring dove (*Turtur risorius*) and the female parent was a hybrid between a male little red ring dove of China (*T. humilis*) and a common ring dove (*T. risorius*).

The abnormalities under consideration occurred in the plumage succeeding the *natal* down, the so-called *juvenal* plumage of Dwight (:00), and they were of three distinct types, belonging respectively to the remiges, the rectrices and the body coverts.

FIG. 1. Dorsal view of secondary from left wing of hybrid pigeon having abnormal plumage. × 1.

1. *The Remiges.* — The remiges all had a transverse band of slightly paler color across the proximal portion of the distal half of the vane (see Fig. 1). The shaft, barbs and barbules were all more sparsely pigmented where this band occurred, and some of the barbules were represented only by stubs. This condition of the barbules is particularly noticeable at the proximal ends of the barbs whose insertions on the shaft occur in this region, and it will be considered in the description of abnormalities in the rectrices. The vane was also slightly narrower at this point. The position and character of this band were uniform in all of the remiges, though there were very slight variations in its paleness.

2. *The Rectrices.* — The rectrices were also crossed by a transverse band in a position similar to that noticed in the remiges, but the region is much more abnormal. The loss in pigmentation is greater than was the case in the remiges, and

there is an area of almost complete absence of normally formed barbules (Fig. 2). The barbules of both the distal and the proximal rows in this region were represented only by stubs of the proximal portions, and the stubs in the distal row were much shorter than those of the proximal row (Fig. 3). To the unaided

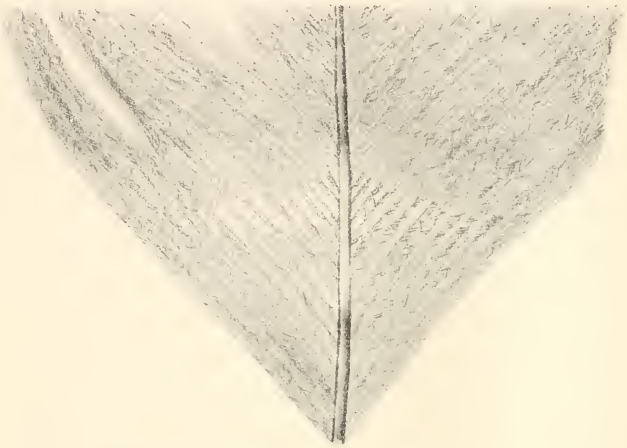


FIG. 2. Dorsal view of the abnormal region in one of the rectrices, the fourth from the left side of the tail. × 6.

eye the vane appears loose in texture at this point and, as in the remiges, it is slightly narrower here.

It has been impossible to determine the histogenesis of these barbule stubs, but it seems to the writer unlikely that they represent the entire structure produced in the feather germ. The occurrence of barbules of normal appearance among the stubs with no intermediate conditions and the irregular fractured appearance of the distal ends of the stubs point strongly towards the conclusion that a distal portion has been broken away. There are a number of species of birds which normally have feathers with the barbules broken off at certain fairly definite points near their proximal ends in the distal portions of the more distal barbs as has been observed by Meves ('55), Chapman ('96), Dwight (:00),

and others, including the writer. The barb itself may be broken near the distal end according to Meves and Chapman. In these instances, the breaking is believed to occur some weeks or months after the feather was fully developed. In the case of these abnormal feathers, however, nothing is known as to the time when the barbules may have been broken, but the condition was observed only a few days after the feathers had emerged from the sheath enclosing the feather germ.

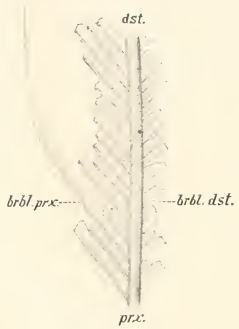


FIG. 3. Dorsal view of a portion of a barb bearing barbule stubs from the left side of the shaft in the region represented in Figure 2. × 74. *brbl. dst.*, distal barbules; *brbl. prx.*, proximal barbules; *dst.*, distal end of portion of barb figured; *prx.*, proximal end of portion of barb figured.

The Body Coverts.—The most striking type of abnormality appeared in the body coverts, and it is very different from what was seen in the remiges and rectrices.

A normal body covert of the juvenal plumage from another hybrid is shown in Fig. 4. The downy portion, *i. e.*, the part where the barbules are long and with rudimentary barbicels is represented diagrammatically. The barbs or portions of barbs bearing shorter barbules with hooked barbicels usually, in the distal row, are indicated semi-diagrammatically by lines radiating from the shaft, but no attempt has been made to represent the true number of the barbules. It will be seen that by far the larger portion of the vane of such a feather is downy.

An abnormal feather from a corresponding region on the back of the bird under consideration is represented in Fig. 5. Almost the whole feather appears downy and there is a strange tassel-like appendage to the distal end of what may be called the main body of the feather. With the aid of a microscope, one finds that two or more of the more distal barbs are fused at their distal ends into a horny, unpigmented, irregularly-shaped cylinder (Fig. 5, *cyl.*), which branches at its distal end into several downy barbs and a median shaft that itself bears barbs on either side in

the usual manner. The shaft of the main portion of the feather is not continuous, however, with the shaft of the distal appendage. The distal ends of the barbs nearest those actually fused with the horn cylinder have strips of horn tissue (Fig. 5, *a*.), which seem to have been torn loose from the cylindrical mass with which they were probably fused in the feather germ.

Near the points where the barbs fuse with this horn cylinder, the barbules are less highly differentiated. The most distal barbules are comparatively irregular strips of keratin which may

FIG. 4. FIG. 5.

FIG. 4. Dorsal view of a normal body covert from the back immediately anterior to the upper tail coverts. × 2.

FIG. 5. Dorsal view of an abnormal body covert from a region corresponding to that furnishing the feather shown in Figure 4. *cyl.*, horn cylinder; *a*, position of a fragment torn from horn cylinder. × 2.

be fused at their distal ends with the cylindrical mass, as can be seen in Fig. 6 where two barbs and a barbule are shown fusing with a strip of tissue torn from the cylinder.

Discussion and Conclusions. — It is significant that these abnormalities occur at uniform distances from the distal ends of the feather throughout the whole plumage, and it seems reasonable to conclude that the conditions responsible for the abnormalities were constitutional, and affected the germs of all the feathers

simultaneously, though in three different degrees of intensity. The length of the period during which these conditions obtained is, presumably, approximately that required for the differentiation of a portion of the shaft equal to the band shown in Fig. 1.

Professor Whitman tells me that he once had a young common domestic dove which developed similarly abnormal feathers. This bird was underfed during the period when the *juvenal* plumage was developing. I am inclined to accept Professor Whitman's suggestion that in both cases the abnormalities are due to malnutrition. The effects upon the remiges and rectrices were comparatively slight, and appear simply as a weakening in pigmentation and structure, but the body coverts were strikingly modified.

FIG. 6. Dorsal view showing fusion of two barbs and a barbule into a strip from the cylinder of undifferentiated cornified tissue which connects the distal appendage shown in Fig. 5 with the main body of the feather. × 49.

The horn cylinder (Fig. 5, *cyl.*) is just such a structure as one would expect to find if the processes of differentiation in the fundament of the barbs and barbules, which have been described by Davies ('89), Strong (:02), and others were to be arrested temporarily. The shaft, barbs and barbules are developed in apartments of a cylindrical mass of epithelium surrounding a central dermal papilla, and differentiation takes place from the proximal end distally. Epithelial cells proliferated at the proximal end of the feather germ push the differentiating epidermal cylinder distally, and cornification takes place some distance from the proximal end. If the differentiation, but not the cornification, should be omitted, a simple cylinder of cornified tissue such as we see in the abnormal body coverts would result. The appearances at either end of the horn cylinder undoubtedly represent the end of differentiation distally, and at the proximal end (Fig. 6) the resumption of differentiation of the normal feather elements.

I wish here to express my thanks to Professor Whitman for the material described in this paper and for courtesies received in connection with the investigation.

THE MARINE BIOLOGICAL LABORATORY,
WOODS HOLL, MASS., September 4, 1902.

BIBLIOGRAPHY.

Chapman, F. M.

'96 On the changes of Plumage in the Snowflake (Plectrophenax nivalis). Bull. Am. Mus. Nat. Hist., Vol. 8, pp. 9–12, 2 text figures.

Davies, H. R.

'89 Die Entwicklung der Feder und ihre Beziehungen zu anderen Integumentgebilden. Morph. Jahrb., Bd. 15, Heft 4, pp. 560–645, Taf. 23–26.

Dwight, J., Jr.

: 00 The Sequence of Plumages and Moults of the Passerine Birds of New York. Annals N. Y. Acad. Sci., Vol. 13, No. 1, pp. 73–360, Pls. 1–7.

Meves, W.

'55 Ueber die Farbenveränderung der Vögel durch und ohne Mauser. Jour. für Ornith , Bd. 3, pp. 230–238, Taf. 2 and 3. Translated from the Oeversigt of K. Vet.-Akad. Förhandl. (Stockholm), 1854. Nr. 8.

St ne, W.

'96 The Molting of Birds with special Reference to the Plumages of the Smaller Land Birds of Eastern North America. Proc. Acad. Nat. Sci. of Philadelphia, pp. 108–167, 2 text figures, Pls. 4–5.

Strong, R. M.

: 02 The Development of Color in the Definitive Feather. Bull. Mus. Comp. Zoöl., Vol. 40, No. 3, pp. 147–185. Pls. 1–9

MATURATION, NATURAL DEATH AND THE PROLONGATION OF THE LIFE OF UNFERTILIZED STARFISH EGGS (ASTERIAS FORBESII) AND THEIR SIGNIFICANCE FOR THE THEORY OF FERTILIZATION.

JACQUES LOEB,

HULL PHYSIOLOGICAL LABORATORY, UNIVERSITY OF CHICAGO.

I. Introduction.

I have pointed out in my earlier publications that fertilization of the egg serves to prolong the life of the egg.[1]

The mature unfertilized egg dies in a comparatively short time. Because of this fact the egg becomes of importance as an object of experiment, to study the question of natural death and prolongation of life. For by no means has it been decided that there is a "natural" death. We only know that with an increase in age a critical period is reached in which every living organism dies under the influence of conditions which do not affect a younger organism. It may, therefore, be of interest that we are able to show, as I believe, that a critical period exists in the life of many eggs in which they die a "natural" death, and that the life of the eggs can, during this period, be saved or lengthened only through various external conditions.

The egg of the starfish (*Asterias Forbesii*) serves as a very favorable object of experiment in the study of this question. When removed from the ovary this egg is generally "immature," but as soon as it comes in contact with sea water it begins to "maturate."

Morphologically, the immature state is characterized by a very large, plainly visible nucleus.[2] The process of maturation consists

[1] Loeb, J., *American Journal of Physiology*, Vol. IV., 1901; Loeb and Lewis, *ibid.*, Vol. VI., 1902.

[2] The recent beautiful experiments of Delage have shown that, beside these visible changes in the nucleus, chemical, but morphologically invisible, changes also occur in the protoplasm. Delage, Études expérimentales sur la maturation cytoplasmique et sur la parthénogenèse artificielle chez les Échinodermes, *Arch. de Zoologie expériment.*, Vol. IX., 1901.

morphologically in this, that the nucleus becomes invisible and the polar bodies are thrown out.

This process is completed within one or two hours after the eggs are removed from the ovaries and placed in sea water. Only when maturation is complete is it possible to cause the egg to develop through the addition of sperm or through the physical and chemical agencies that have been described by me, Delage, Mathews, and Greeley.

II. The Natural Death of the Mature Unfertilized Starfish Eggs.

The living eggs of *Asterias* are light yellow in color and homogeneous. They retain this appearance during the process of maturation as long as they are alive. They retain this appearance also when they are made to develop through the entrance of a spermatazoön or through the proper chemical or physical means.

If, however, the mature eggs are not fertilized or do not develop, they die in the course of four to twelve hours, and this process of dying is accompanied by a characteristic change in the color of the egg. The egg becomes at first opaque, then almost black, the homogeneous structure of the protoplasm becomes granular. If such a culture of unfertilized eggs is examined under the microscope after twenty-four hours, two kinds of eggs are found, first, the just described dark, dead eggs *which are mature,* and secondly, *living* normally colored *but immature* eggs. For usually not all the eggs that are removed from the ovaries of a starfish maturate at once; many maturate very late, others not at all. It is readily seen *that the immature eggs remain alive for several days until they finally become the prey of bacteria; while the mature eggs become opaque and die in four to twelve hours after maturation has been completed.*

Is the death of the mature but undeveloped egg brought about through internal conditions or through the bacteria contained in the sea water?

A trustworthy way of determining this consists in making sterile culture of the eggs in sea water. This is a relatively simple procedure in the case of starfish. Eight flasks were sterilized, filled with sterilized sea water, and again heated for twenty

minutes on three successive days to 100°C. A female starfish was thoroughly washed externally, an arm was opened, and one of the ovaries removed with sterilized forceps and placed in sterilized sea water. From the thick stream of eggs which at once flowed out of the ovary, a few drops were quickly introduced with a sterilized pipette into each of the sterilized flasks. A second series of eight flasks contained normal sea water, and a few drops of the same eggs were introduced into these flasks also. A third series of flasks were filled with sea water, to each of which were added 2 c.c. of a putrid, foul-smelling culture of old starfish eggs in order to bring about a rapid development of bacteria from the beginning. Each of these flasks also contained eggs from the same culture as those in the sterilized flasks.

That perfect sterilization had been attained in the first eight flasks was proved by the fact that all the flasks remained absolutely clear and cloudless during the course of the experiment, and that three of the flasks which had not been opened are even to-day (after six weeks) absolutely clear, and every egg can be individually recognized. The flasks containing the unsterilized sea water became cloudy already after twenty-four hours, and after two days the eggs had become the prey of bacteria and no individual egg could be recognized. The sterilized flasks which were opened were at all times free from foul odor, while the unsterilized flasks gave off a penetrating stench, often after one, invariably after two days. The microscopic examination of the sea water for bacteria was always negative in the sterilized flasks, always positive in the other flasks. In those flasks to which 2 c.c. of the putrid culture of starfish eggs had been added, bacteria and infusoria were exceedingly numerous from the beginning.

Six hours after the beginning of the experiment one flask of each of the three series was opened and the eggs examined microscopically. The picture was the same in all three flasks. Nearly all the eggs were mature, and a small number of them were opaque or black. But what is of the greatest importance to us is the fact that the percentage of opaque dead eggs was just as great in the sterile culture (if not greater) than in the unsterilized or the infected sea water.

Twelve hours later, that is to say eighteen hours after the

beginning of the experiment, one of the flasks of each of the three cultures was again opened. At this time nearly all the eggs of the sterile culture were opaque or black, and a few were already granular. In the two other cultures an equal percentage of the eggs were opaque. The eggs, therefore, die just as rapidly in the sterilized flasks which are absolutely free from bacteria as in the flasks containing bacteria. Death follows from internal causes, and so rapidly that the few bacteria in the sea water are scarcely able to accelerate the death of the eggs. The eggs have already died from internal causes before the bacteria can attack them in sufficient numbers to threaten their existence.

The flasks which were opened later served only to corroborate what has been said. The experiment was repeated with the same result. Each of the flasks that were opened during the first few days also contained a small number of living transparent eggs. *The latter were, without exception, immature. The experiment, therefore, shows that the mature eggs of starfish die in the course of a few hours, and that the cause of this death cannot be sought in the bacteria of the sea water; and further, that under exactly the same conditions the immature eggs remain alive.*

III. The Chemical Conditions Necessary for Maturation in Starfish Eggs.

Since the eggs of *Asterias* are usually immature in the ovary, but, in part, at least, maturate in the course of one or two hours when introduced into sea water, the suspicion was aroused that some of the substances contained in the sea water brought about the maturation. In order to determine which substance this might be, a series of solutions were prepared having approximately the osmotic pressure of the sea water. The result was so simple that it is not necessary to describe all the experiments here. For it was found that when the eggs are introduced into solutions which contain free hydroxyl ions, maturation soon follows, but that this does not occur in solutions containing no hydroxyl ions. So, for example, the eggs retain their nucleus in a $\frac{5}{8}n$ NaCl solution, or in NaCl solutions to which some potassium or calcium has been added. If, however, .5 to 2 c.c. $n/10$ NaOH is added to each 100

c.c. of such solutions, maturation soon follows; that is to say, the nucleus becomes invisible. Since sea water contains free hydroxyl ions the conclusion is justified that these are one of the causes for the maturation of the starfish egg. It was possible to prove this assumption through further experiments. If a small amount of acid is added to sea water, the free OH ions disappear, and the water becomes acid in reaction (through the addition of 1.5 c.c. or more *n*/10 HCl to 100 c.c. sea water). Immature eggs were introduced directly into sea water to which 1, 2, 3 and 4 c.c. of an *n*/10 HNO_3 solution had been added to each 100 c.c. of sea water. While, as is usual, a large percentage of eggs soon maturated in the normal sea water, maturation did not occur at all in the vast majority or in all the eggs contained in the sea water to which 2 or more c.c. acid had been added. The addition of even 1 c.c. of acid diminishes the number of eggs that maturate. But it is not even necessary to keep the eggs permanently in neutral or acid sea water in order to inhibit maturation. If 4 or 5 c.c. of a *n*/10 HNO_3 solution are added to 100 c.c. sea water, and immature eggs are introduced into such a solution for only about fifteen minutes, relatively few eggs maturate when they are returned to normal sea water. Such acidified sea water does not kill the starfish eggs.

We shall see later that the procedure described here which, when used upon immature eggs, prevents maturation, brings about artificial parthenogenesis when used on mature eggs.[1]

I have, moreover, been able to convince myself of the fact that the eggs which are introduced into acidified sea water in an immature state, can be fertilized by sperm if they finally maturate. It is possibly in harmony with what has just been said that the addition of $NaHCO_3$, or larger amounts of sodium citrate to the sea water accelerates the process of maturation. Free hydroxyl ions are present in the solutions of both substances, and it is possible that their addition to the sea water increases the concentration of the free hydroxyl ions in the sea water.

But the hydroxyl ions are certainly not the only substances in the sea water which favor or cause the maturation of the starfish egg. I soon found that when different specimens of eggs are

[1] Loeb, Fischer, and Neilson, *Pflüger's Archiv*, 1901, Bd. 87.

taken from the same culture, and the percentage of mature eggs is determined, this percentage is subject to the greatest variations. The cause of these variations was soon discovered. For it was found that where the eggs lie together in a heap maturation occurs slowly, but where they lie in a thin layer, maturation occurs quickly. This fact suggested the importance of oxygen for maturation. Where the eggs lie in a heap the appropriation of the oxygen by the superficial layers of eggs prevents the diffusion of the oxygen to those lying deeper.

Experiments were now made in which the oxygen of a small flask containing a small amount of sea water was replaced by hydrogen. When, in such experiments, all the oxygen was entirely removed maturation did not occur in any, or at least the majority of the eggs, in spite of the presence of the hydroxyl ions in the sea water. There are, therefore, at least two substances in sea water which cause or accelerate maturation, oxygen and hydroxyl ions. Possibly other constituents of the sea water are also concerned in the process, but NaCl, Ca, and K have apparently no beneficial effect upon maturation.[1]

It seems, therefore, that the absence of oxygen and hydroxyl ions in the ovaries belongs to the conditions which inhibit maturation of the eggs in the ovary.

IV. The Prolongation of the Life of the Unfertilized Starfish Egg by the Prevention of Maturation.

We have shown above that the mature eggs of a culture of unfertilized starfish eggs die within a short time (which decreases with an increase in temperature), while the immature eggs remain alive a relatively long time. It was necessary now to show that when the maturation of a culture of unfertilized egg of *Asterias* is prevented artificially, the eggs live longer. We begin with the experiment which is technically most simple. The eggs streaming from the ovary are divided into two portions. One portion of eggs is carefully distributed without mechanical agitation, by carefully tipping the vessel, in a thin layer over the bottom

[1] Professor Whitman informs me that the maturation of the eggs of *Clepsine* does not begin until after they are laid. Possibly the oxygen contained in the water is in this case also a necessary condition for maturation.

of the vessel. The vessel must be low and the layer of sea water covering the eggs not too deep, so that the diffusion of oxygen to the eggs can occur with ease. A second portion is introduced with just as great care into a small-calibered glass tube sealed at one end. This glass tube is half filled with eggs, so that one is certain that the lower layers of the eggs in the pipette receive little or no oxygen. It is self-evident that the eggs must be introduced into the tube immediately after being laid. When, after twenty-four hours, the eggs which are distributed over the bottom of the glass dish and which receive a large amount of oxygen are compared with those at the bottom of the glass tube, a striking difference is found between them. The eggs richly supplied with oxygen contain a much larger percentage of mature dead and black eggs than those kept in the lack of oxygen. In the latter the living immature eggs are in the majority, and a part of these maturate when spread out in a thin layer over the bottom of a vessel. These experiments are also well adapted to show that the rapid death of the mature unfertilized sea-urchin eggs is determined through internal conditions and not by the bacteria contained in the sea water. I will cite an example.

One portion of a lot of eggs was spread out in a thin layer over the bottom of a dish; another was heaped in a mass in the same dish. The sea water was the same in both cases. The first portion of eggs maturated in a few hours and were, in less than twelve hours, opaque and dead, while the water was still absolutely clear and without odor of putrefaction. After twenty-four hours the water became putrid and contained many bacteria. Even after three days, when the water was exceedingly foul and cloudy, a portion of the eggs which had lain in a heap, that is to say, without oxygen, *were immature and living*. They were introduced into fresh water and spread out into a thin layer. They maturated and developed into swimming larvæ upon the addition of sperm. It is self evident of course that even immature eggs finally become the prey of bacteria, and so go to pieces in the sea water.

The same experiment can be made in a somewhat more complicated way with pure oxygen and hydrogen. The freshly laid eggs

of a starfish were distributed into two series of eight flasks. The one series of flasks was connected with a hydrogen generator; the other with a tank containing pure oxygen. Before the beginning of the experiment all the air in one of the series of flasks was driven out by the current of hydrogen. During the course of the experiment a vigorous current of hydrogen was maintained. Both series of flasks contained freshly laid immature eggs of *Asterias*. The experiment lasted three days, and from time to time a flask was removed and its contents examined. The eggs which had been exposed to the current of oxygen maturated just as rapidly and as numerously as those in ordinary sea water, and the mature eggs soon died. In the current of hydrogen maturation did not occur in the majority of the eggs, and these remained alive. In the hydrogen cultures a rapid development of bacteria occurred while in the oxygen cultures this occurred to a small degree.[1]

Treatment with acids which, as we have shown above, prevents the maturation of the eggs (without killing them) also prevents their death and disintegration.

Eggs which, without having been in contact with pure sea water, are introduced for ten or fifteen minutes into 100 c.c. sea water plus 4 c.c. *n*/10 HCl maturate very slowly or not at all when they are returned to normal sea water. They also retain, as long as they are immature, the transparent, normal appearance of living eggs until they finally become the prey of bacteria.

It seems to follow from these experiments that the same processes which underlie the maturation of starfish eggs also lead to their death (if they are not inhibited through circumstances which we designate by the term fertilization). I tried to see, now, whether it was also possible to maintain the life of the *mature* egg through lack of oxygen. I indeed obtained in a few cases positive results in this direction. The eggs of a starfish were spread in a thin layer over the bottom of a dish. After three hours seventy-five per cent. of the eggs were maturated. A portion of the mature eggs were carefully introduced into the

[1] Care must be taken in these experiments that the air is thoroughly removed from the sea water in the hydrogen flasks before the eggs are introduced into them. Of course the hydrogen apparatus must also be free from air.

glass tube described above, in which the deeper layers suffered from lack of oxygen. A second portion was introduced into a small flask through which a steady stream of pure oxygen was passed. On the following morning, that is to say, fifteen hours after the eggs were brought into the atmosphere of pure oxygen, the various portions of the eggs were examined. The eggs introduced into the current of oxygen showed in one vessel *98 per cent. mature and dark, dead eggs and 2 per cent. immature living* eggs. The eggs which had remained in normal sea water contained, as before, about 75 per cent. mature eggs, all of which, however, were black and dead, with the exception of a few eggs which had begun to divide,[1] and were living.

The immature eggs were also still living. Upon the other hand, the eggs which had been left in the glass tube in absolute or relatively high lack of oxygen, were nearly all living! This observation seems to show that the same processes which lead to the maturation of the egg bring about its death if they are not inhibited at the right time. In this way the process of fertilization becomes a life-saving or life-prolonging act.

V. Do these Facts Hold for Other Forms?

The question of the relation between maturation and natural death can be studied most beautifully in the starfish egg because it is possible to obtain it in an immature condition, and because maturation follows very rapidly. With sea-urchin eggs conditions are much less favorable, since the egg maturates within the ovary, and since it is difficult to obtain immature eggs during the spawning season. I have, therefore, been unable to discover which chemical factors determine the maturation of the sea-urchin egg, and to decide whether the same circumstances cause the death of the sea-urchin egg that bring about the death of the starfish egg; and whether the life of the sea-urchin egg can be prolonged through a prevention of these circumstances. In an indirect way Lewis and I attempted to answer this question last year, when we assumed that the destructive processes which bring about the

[1] This cleavage was possibly brought about through mechanical agitation; I had repeatedly shaken the dish to facilitate the introduction of oxygen into the sea water.

death of the unfertilized egg are enzymatic (autolytic?) processes which can be inhibited through poisons such as KCN.[1]

We did in fact succeed in showing that the addition of a small amount of KCN to the unfertilized starfish eggs markedly lengthens their life. Even after seven days such eggs can be fertilized as soon as they are returned to normal sea water. We also pointed out that, because of the well-known bactericidal properties of potassium cyanide, the experiments on sea-urchin eggs were not in themselves decisive and so began experiments on starfish eggs[2] which, however, we were not able to complete at that time. In dealing with eggs which are as long lived as sea-urchin eggs a great development of bacteria in normal sea water can not be prevented, since a few of the eggs always die and so serve as an excellent culture medium for the further development of bacteria. It need, therefore, surprise no one that the unfertilized eggs of sea-urchins, as I was able to show this year, live in sterile sea water for five days, or possibly longer, while they die much earlier in ordinary sea water (about two days). The very fact that the eggs of sea-urchins are found mature in the ovary indicates that they are able to live a considerable time after maturation and that they differ in this respect from the starfish egg.

It is, however, a fact that in the same sea water the fertilized and developing sea-urchin eggs live longer than the unfertilized eggs.

It almost seems as if in certain of the higher animals there are eggs which develop only when they are fertilized immediately after leaving the ovary. Under the direction of Professor C. O. Whitman, Harper has shown that the eggs of pigeons are fertilized the moment they leave the ovary. The sperm lives in a gelatinous mass upon the surface of the ovaries,[3] so that provision is made for the necessary contact between sperm and egg. This also does away with the difficulty which many have found in

[1] Loeb and Lewis, *American Journal of Physiology*, Vol. VI., 1902.

[2] Loeb and Lewis, *American Journal of Physiology*, Vol. VI., 1902.

[3] Spermatozoa are in general much longer lived than eggs, even though great differences exist in this regard in different animals. In the spermatic vesicles of the queen bee spermatozoa are believed to remain more than a year after copulations.

explaining how the spermatazoön finds its way to the egg in animals in which fertilization occurs within the body. Definite directive forces are clearly not necessary, since a portion of the spermatazoa must reach the ovary, through their ciliary motion, by way of the uterus and Fallopian tubes. Experiments similar to those made by Harper upon pigeons must yet be made upon mammals. Yet there seems to be no doubt that the mammalian egg of many species is also fertilized before it reaches the uterus. Cases of extra-uterine pregnancy also point to the possibility that fertilization may occur at the surface of the ovary.

VI. The Prolongation of Life and the Theory of Fertilization.

Our experiments seem to have proved that the mature unfertilized starfish egg dies within a few hours through internal changes but that the process of fertilization saves the life of the egg. This is true, not only of the fertilization of the starfish egg by spermatozoa, but also for the chemical fertilization through hydrogen ions. Mr. Neilson succeeded this year in keeping the parthenogenetic larvæ of starfish alive much longer than has thus far been the case (over thirty days), and Dr. Fischer was able to accomplish the same for the larvæ produced osmotically from unfertilized sea-urchin eggs. It is therefore possible that the chemical or osmotic fertilization of these eggs can give rise to as long-lived larvæ as the fertilization of the egg through sperm.

But how does the spermatozoön or the physical and chemical means substituted for it, save the life of the egg and why does the mature egg die when it is not fertilized by sperm or artificial means? I believe that the answer lies in this, that the fertilizing agencies accelerate metabolic processes in the egg which, before fertilization, went on only slowly. After fertilization by sperm or by the chemical or physical means substituted for it, the egg divides and grows, which it did not do before fertilization occurred. Growth is inconceivable without a preponderance of synthetical over hydrolytical processes. I believe it possible that the determining factor in the chemical forces set in motion within the egg through fertilization consists in this that the synthetical processes in the egg are accelerated. If these processes are not inaugurated

or accelerated the egg dies. The wasting of the body in old age also indicates a decrease in synthetical processes. Whether the second critical period occurring in old age is similar to the critical period of the egg cannot yet be determined. Yet it is not impossible that the question of the prolongation of life at this period should pass over into the question of the possibility of accelerating synthetical processes.

We, therefore, come to the conclusion that fertilization accelerates a series of chemical changes (syntheses?) in the egg which do not occur sufficiently rapidly without spermatic, chemical or osmotic fertilization in the eggs of the majority of animals. But why does the mature egg die when these processes are not accelerated, and why does it remain alive before it maturates? The egg must often exist for years in the immature condition in the ovary. In answer I can only suggest *that the processes underlying maturation are at least in some form of a destructive nature (one might think of autolytic processes) which the egg cannot withstand for an indefinite length of time without dying.* In many eggs the velocity of these destructive (autolytic?) processes may be greater than in others and this may determine the differences in the velocity with which the mature, unfertilized egg dies. It is in harmony with this view that when maturation is prevented, or the mature egg is put under condition which inhibit the process of maturation or the chemical processes underlying it, that the life of the egg is lengthened. Lack of oxygen or the addition of an acid work in this way in the case of starfish eggs; a slight addition of potassium cyanide in the case of starfish and sea-urchin eggs. But since all of these substances injure the eggs indirectly and do not entirely do away with the destructive (autolytic?) processes occurring within the egg, life is not prolonged to the same extent by these means as by fertilization in which case life is prolonged not only through an inhibition of the destructive but also through an acceleration of the synthetical processes.

That the chemical processes which underlie maturation are not identical with those which bring about fertilization seems to be supported by the observation made above, that the same means —the treatment with acid—which causes the mature egg to

develop and live beyond the bipinnarian stage, inhibits the maturation of the immature egg. When the *mature* unfertilized eggs of a starfish are introduced for fifteen to sixty minutes into a mixture of 100 c.c. sea water plus 3 c.c. *n*/10 HCl ninety per cent of the eggs can, under favorable conditions, develop into larvæ. If, however, the eggs are introduced into such a solution for the same length of time *before maturation*, the maturation of the eggs is prevented either permanently or for a long time.[1] The difference is still more striking when the eggs are kept for a shorter time in a mixture of 100 c.c. seawater and 5 c.c. *n*/10 HCl. This shows that acid affects the process of development and the process of maturation in opposite or at least different ways.

We must now raise the question, How does the behavior of naturally parthenogenetic eggs, such as the eggs of bees harmonize with these ideas?

In naturally parthenogenetic eggs it seems as if the processes which underlie maturation pass over into those underlying development. But it is possible that this is only apparently the case, and that in reality it so happens that in the processes underlying maturation a metabolic product is formed in the parthenogenetic animals which favors the processes of development. We know that an exceedingly small amount of hydrogen ions suffices to bring about development in unfertilized starfish eggs; that an exceedingly small amount of calcium causes the unfertilized eggs of *Amphitrite* to develop; and that a trace of potassium ions brings about the development of unfertilized *Chætopterus* eggs.[2] It is entirely possible that the specific ions or other substances necessary to start the development of the eggs of the bee are formed within the egg itself through the chemical changes taking place during or after maturation, and that without the formation of these substances development is impossible. In the case of sea-urchins and starfish eggs one might also believe that the processes of maturation and the processes of development pass over into each other. For it has often been observed that the

[1] The eggs which finally maturate in spite of the previous treatment with acid often begin to cleave when maturation is complete and develop into larvæ, while the control eggs kept in normal sea water do not develop.

[2] Loeb, Fischer and Neilson, *l. c.*

unfertilized eggs of these forms after having resided in "normal" sea water for about twenty-four hours begin to cleave shortly before death. This cleavage, however, never goes beyond the two- or four-celled stage. This might be explained by the fact that the eggs begin to die at this time. After I had found this year that the eggs of sea-urchins can still be fertilized after a residence of five days in sterilized sea water (at summer temperature), I decided to study this question of spontaneous cleavage somewhat more closely. If it were true that individual sea-urchin eggs begin to cleave in ordinary sea water after about twenty hours, and cease to develop any further only because they soon die, it would be expected that many or all should cleave when kept alive four or five days, and that a number of them should reach a fairly advanced stage of development. A lot of sea-urchin eggs were distributed into a series of flasks containing sterile sea water. One of the flasks was opened every morning and a careful search was made for developed eggs.

In the course of five days I never found a single divided egg either in the two-celled stage or in later stages of development. It is possible that during the last days of the experiment a few eggs divided, and that the cleavage cells fell apart. Lewis and I found last year that when eggs are fertilized forty-eight or more hours after their removal from the ovaries they form no membrane and the cleavage cells fall apart. I have corroborated this fact this year. Usually more than one embryo develops from such an egg, because the cells drop apart. I kept this fact in mind and will not disavow that a few small eggs were present, which perhaps represented only half the mass of an ordinary egg. But nearly all the eggs were of normal size, and since small eggs are occasionally found even under normal conditions, the experiment shows that in sea-urchin eggs also the processes of maturation are not continuous with those of cleavage, and that entirely different conditions which we can bring about through the abstraction of water or the entrance of a spermatozoön are necessary that division may occur. It cannot be urged that the sterilized water perhaps prevented the cleavage. When at the conclusion of the experiment these same eggs were fertilized in sterilized water by adding a drop of sperm, they developed to the pluteus stage in

sterile sea water. I, therefore, consider it possible that where authors describe a cleavage of the unfertilized sea-urchin eggs in "normal" sea water, that the sea water or the eggs had in reality suffered some change which had escaped the notice of the observers. One might think of evaporation and increase in the osmotic pressure of the sea water. A very slight increase in the osmotic pressure of the sea water is sufficient to cause the sea-urchin egg to divide into two cells in the course of twenty hours. One might also think of a change in the sea water brought about by the putrefaction of the dead eggs. Finally it is possible that a substance is perhaps formed (for example, an acid) in the dying eggs which brings about a single cleavage.

The relations which exist between maturation and natural death upon the one hand, and fertilization and the prolongation of life upon the other, lead us to the conclusion that a "fertilization" must perhaps come to pass in every egg, even in those naturally parthenogenetic. Only according to our idea, the act of fertilization is not identical with the morphological process which is designated fertilization. It is rather a chemical or a physico-chemical act which accelerates certain (synthetical?) metabolic changes in the egg, which occur in the egg under ordinary conditions also, only much too slowly. The difference between naturally parthenogenetic eggs and the eggs which must be fertilized before they can develop consists perhaps in this, that to the latter the catalytically working substance or complexus of conditions must be added from the outside in order to accelerate the synthetical (?) processes, while in the naturally parthenogenetic eggs these substances are formed within the eggs (possibly in conjunction with the processes of maturation).

The connection between the prolongation of life and fertilization clearly points out that every purely morphological theory of fertilization is incomplete and that a correct theory of this process must have a physco-chemical basis. The means of reaching this basis I see in further attempts at causing development of unfertilized eggs through unequivocal physical and chemical means.

VII. Conclusions.

1. Our observations and experiments seem to show that in the same sea water and under otherwise identical conditions, *mature but unfertilized* starfish eggs soon die, while immature as well as mature but *fertilized* eggs live longer.

2. It seems certain that the rapid death of the mature unfertilized starfish eggs is determined by internal conditions connected with maturation and not by the bacteria contained in the sea water. The proofs for this are: First, mature eggs die just as rapidly in sterilized sea water free from bacteria as in unsterilized water, and secondly, when maturation is prevented artificially the eggs may continue to live in water containing many bacteria.

3. We have shown that oxygen and free hydroxyl ions accelerate the maturation of starfish eggs; that lack of oxygen and a neutral or faintly acid reaction of the sea water inhibit or prevent maturation. The fact that the eggs which remain immature in the ovaries of the starfish maturate when brought into sea water seems to find its explanation in part at least through this.

4. When the maturation of starfish eggs is prevented artificially through lack of oxygen or the addition of an acid to the sea water they remain alive much longer than when they maturate. The eggs in which maturation has already begun or has just been completed seem also to be saved from rapid death by these means.

5. It seems to follow from these facts that the same chemical processes do not necessarily underlie the process of maturation and the process of fertilization. Fertilization by spermatozoa, chemical or physical agencies lengthens the life of the egg, while the changes following the maturation of the egg lead, sooner or later, to death (through autolysis?). It is in harmony with what has been said that the same treatment with acid which brings about artificial parthenogenesis in mature starfish eggs inhibits the process of maturation when used upon immature starfish eggs.

7. These facts corroborate a suggestion which I have made before, that the fertilizing action of the spermatozoön consists in this, that it carries into the eggs substances which accelerate the course of certain (synthetical?) processes in the egg. Such an acceleration might, for example, be brought about through certain ions (for example, the hydrogen ions of nucleic acid), yet the

possibility that such catalytic effects might also be brought about through enzymes or other substances is of course not shut out. Yet this fact must be considered: that we have been able to produce artificially *normal* embryos *capable of development* through ions, while the careful experiments of Gies conducted in my laboratory in which he attempted to find the same to hold for enzymes have thus far failed.

In conclusion I wish to thank my assistant, Mr. Neilson, for the assistance which he rendered me in these experiments.

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